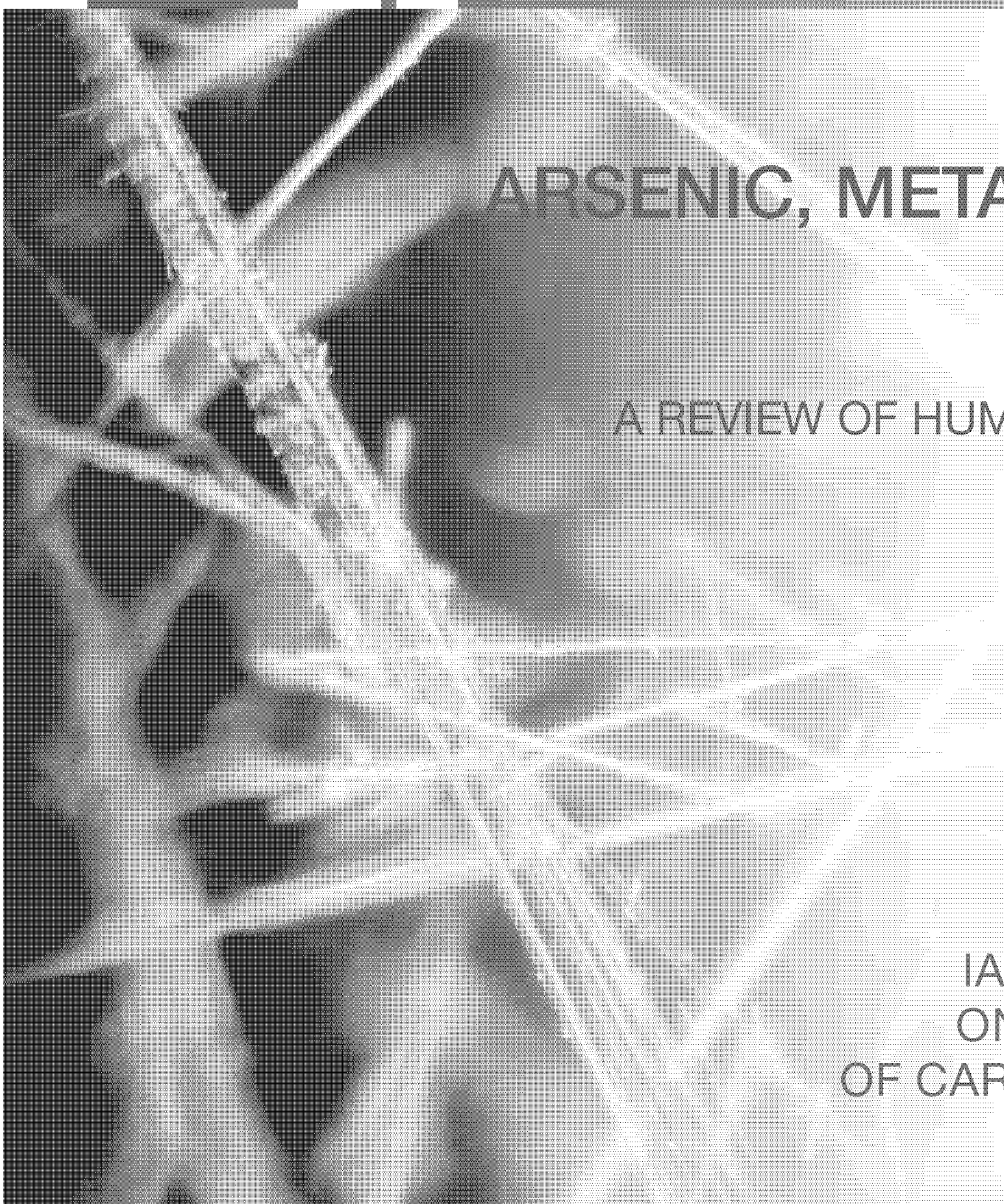
Exhibit 93, part 1

IARC MONOGRAPHS



ARSENIC, METALS, FIBRES, AND DUSTS

VOLUME 100 C

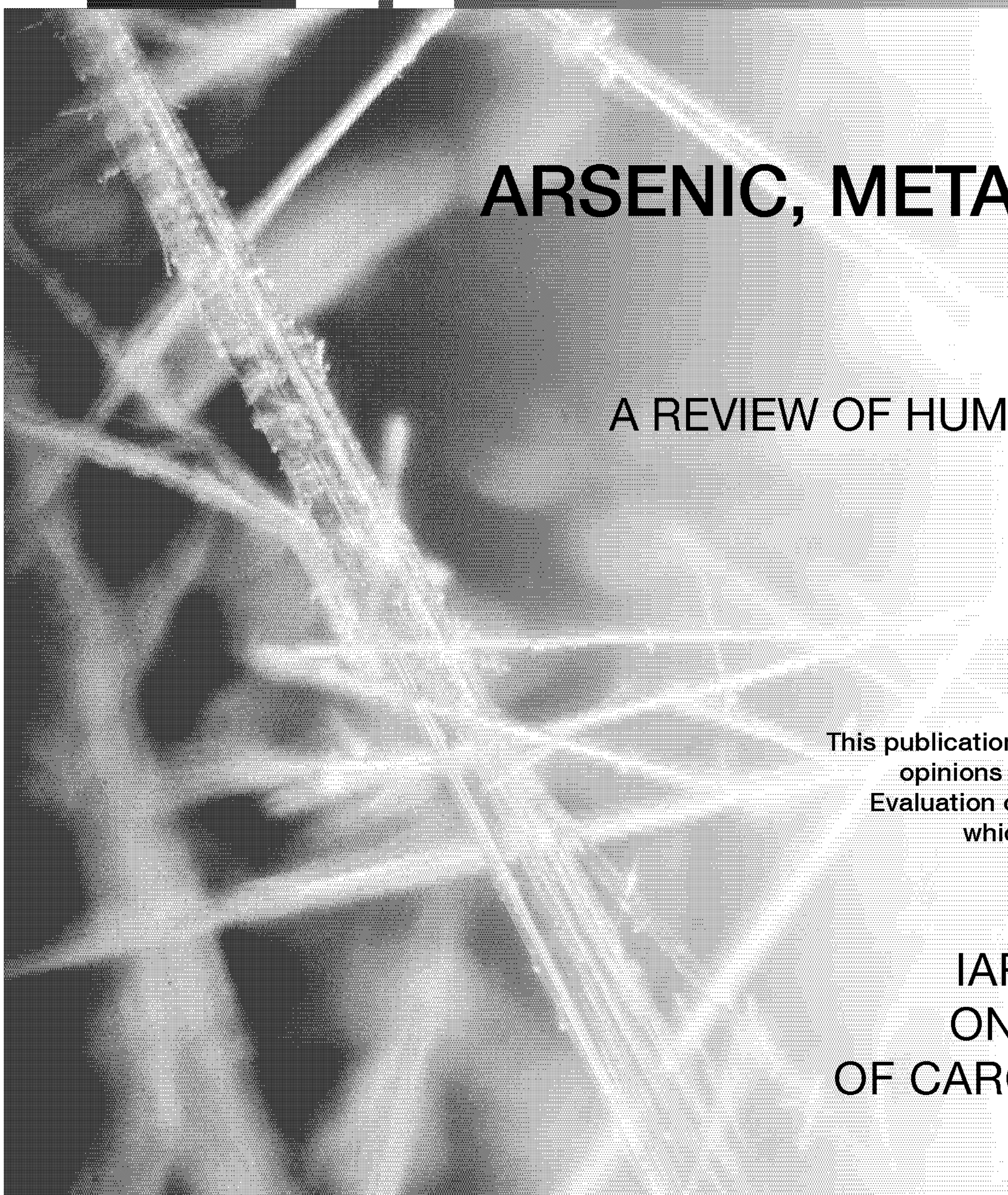
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



ARSENIC, METALS, FIBRES, AND DUSTS

VOLUME 100 C
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 17-24 March 2009

LYON, FRANCE - 2012

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields; and to indicate where additional research efforts are needed. The lists of IARC evaluations are regularly updated and are available on the Internet at http://monographs.iarc.fr/.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the Health, Safety and Hygiene at Work Unit of the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities, and since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the U.S. National Cancer Institute, the U.S. National Institute of Environmental Health Sciences, the U.S. Department of Health and Human Services, or the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities.

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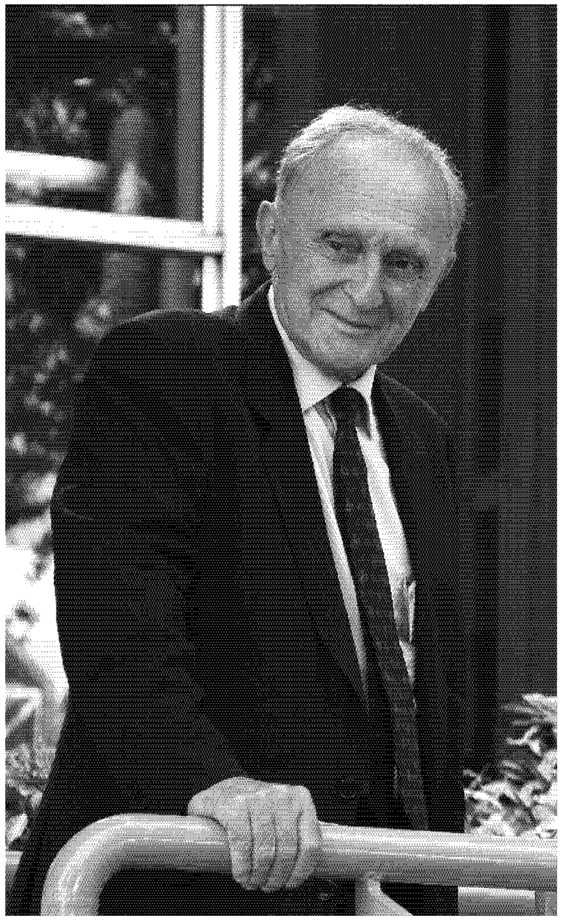

Lorenzo Tomatis (1929-2007)
Founder of the *IARC Monographs* Programme

Lorenzo Tomatis, MD, with other colleagues knowledgeable in primary prevention and environmental carcinogenesis, perceived in the 1960s the growing need to objectively evaluate carcinogenic risks by international groups of experts in chemical carcinogenesis. His vision and determination to provide a reliable source of knowledge and information on environmental and occupational causes of cancer led to his creating the *IARC Monographs* Programme for evaluating cancer risks to humans from exposures to chemicals. The first meeting, held in Geneva in December 1971, resulted in Volume 1 of the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man [1972], a series known affectionately since as the "orange books". As a champion of chemical carcinogenesis bioassays, Tomatis defined and promoted the applicability and utility of experimental animal findings for identifying carcinogens and for preventing cancers in humans, especially in workers and children, and to eliminate inequalities in judging cancer risks between industrialized and developing countries. Tomatis' foresight, guidance, leadership, and staunch belief in primary prevention continued to influence the *IARC Monographs* as they expanded to encompass personal habits, as well as physical and biological agents. Lorenzo Tomatis had a distinguished career at the Agency, arriving in 1967 and heading the Unit of Chemical Carcinogenesis, before being Director from 1982 to 1993.

Volume 100 of the *IARC Monographs* Series is respectfully dedicated to him.

(photo: Roland Dray)

CONTENTS

NOTE TO THE READER

The term ‘carcinogenic risk’ in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word ‘risks’ in the title.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a *Monograph* does not mean that it is not carcinogenic. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Section of IARC Monographs, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the *Monographs* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Section of IARC Monographs, so that corrections can be reported in future volumes.

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PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended '...that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.' The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase 'of chemicals' was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 (Stewart & Kleihues, 2003). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad hoc Advisory Groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991; Vainio *et al.*, 1992; IARC, 2005, 2006).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term 'agent' refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as causation of, and susceptibility to, malignant disease become more fully understood.

A cancer 'hazard' is an agent that is capable of causing cancer under some circumstances, while a cancer 'risk' is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word 'risks' in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

The Preamble continues the previous usage of the phrase 'strength of evidence' as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991; Vainio *et al.*, 1992; IARC, 2005, 2006; see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged

on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose–response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–response relationship. A *Monograph* may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose–response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (http://monographs.iarc.fr). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate

or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

(a) The Working Group

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b) Invited Specialists

Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies

Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) Observers with relevant scientific credentials

Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at http://monographs.iarc.fr).

(e) The IARC Secretariat

The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano *et al.*, 2004).

The names and principal affiliations of participants are available on the *Monographs* programme web site (http://monographs.iarc.fr) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano *et al.*, 2005).

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme web site (http://monographs.iarc.fr) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

For most chemicals and some complex mixtures, the major collection of data and the preparation of working papers for the sections on chemical and physical properties, on analysis, on production and use, and on occurrence are carried out under a separate contract funded by the US National Cancer Institute. Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme web site soon after the meeting.

B. SCIENTIFIC REVIEW AND EVALUATION

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

Exposure data
Studies of cancer in humans
Studies of cancer in experimental animals
Mechanistic and other relevant data
Summary
Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

(a) General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

(b) Analysis and detection

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

(c) Production and use

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production,

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with date and place. For biological agents, the epidemiology of infection is described.

(e) Regulations and guidelines

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccination and therapy, are described.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

(a) Types of study considered

Several types of epidemiological study contribute to the assessment of carcinogenicity in humans — cohort studies, case–control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series of cancer in humans may also be reviewed.

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

First, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than those with national rates. Internal comparisons of

frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Third, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) (Greenland, 1998).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal

relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio *et al.*, 1992; Toniolo *et al.*, 1997; Vineis *et al.*, 1999; Buffler *et al.*, 2004). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) Criteria for causality

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and

coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first that the studies meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio *et al.*, 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate

(e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. OECD, 2002).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

(a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo

transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship (Hoel et al., 1983; Gart *et al.*, 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto *et al.*, 1980; Gart *et al.*, 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman *et al.*, 1994; Dunson *et al.*, 2003).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls,

particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals (Haseman *et al.*, 1984; Fung *et al.*, 1996; Greim *et al.*, 2003).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) Toxicokinetic data

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose–response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) Data on mechanisms of carcinogenesis

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal hormones and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap–junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis (Vainio *et al.*, 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system in vitro affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published (Montesano *et al.*, 1986; McGregor *et al.*, 1999).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of

greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals in vivo indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated in vivo provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986; McGregor *et al.*, 1999).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. Capen *et al.*, 1999).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. ‘Physical agents’ may also be considered to comprise foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) Other data relevant to mechanisms

A description is provided of any structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

(e) Data on other adverse effects

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be found on the *Monographs* programme web site (http://monographs.iarc.fr).

(a) Exposure data

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) Cancer in humans

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose–response and other quantitative data are also summarized.

(d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and

the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

6. Evaluation and rationale

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. The strength of the mechanistic evidence is also characterized.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of the degree of evidence is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of the degree of evidence.

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:** The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

***Limited evidence of carcinogenicity*:** A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence of carcinogenicity*:** The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

***Evidence suggesting lack of carcinogenicity*:** There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In

addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity**:** The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

Limited evidence of carcinogenicity**:** The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

Inadequate evidence of carcinogenicity**:** The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

Evidence suggesting lack of carcinogenicity**:** Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics,

physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental

animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is probably carcinogenic to humans.

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is possibly carcinogenic to humans.

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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GENERAL REMARKS

Part C of Volume 100 of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* contains updated assessments of arsenic, metals, fibres, and dusts that were first classified as *carcinogenic to humans (Group 1)* in Volumes 1–99.

Volume 100 – General Information

About half of the agents classified in Group 1 were last reviewed more than 20 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent epidemiological studies and animal cancer bioassays have demonstrated that many cancer hazards reported in earlier studies were later observed in other organs or through different exposure scenarios. Much can be learned by updating the assessments of agents that are known to cause cancer in humans. Accordingly, IARC has selected *A Review of Human Carcinogens* to be the topic for Volume 100. It is hoped that this volume, by compiling the knowledge accumulated through several decades of cancer research, will stimulate cancer prevention activities worldwide, and will be a valued resource for future research to identify other agents suspected of causing cancer in humans.

Volume 100 was developed by six separate Working Groups:

Pharmaceuticals

Biological agents

Arsenic, metals, fibres, and dusts

Radiation

Personal habits and indoor combustions

Chemical agents and related occupations

Because the scope of Volume 100 is so broad, its *Monographs* are focused on key information. Each *Monograph* presents a description of a carcinogenic agent and how people are exposed, critical overviews of the epidemiological studies and animal cancer bioassays, and a concise review of the toxicokinetic properties of the agent, plausible mechanisms of carcinogenesis, and potentially susceptible populations, and life-stages. Details of the design and results of individual epidemiological studies and animal cancer bioassays are summarized in tables. Short tables that highlight key results appear in the printed version of Volume 100, and more extensive tables that include all studies appear on the website of the *IARC Monographs* programme (http://monographs.iarc.fr). For a few well-established associations (for example, tobacco smoke and human lung cancer), it was impractical to include all studies, even in the website tables. In those instances, the rationale for inclusion or exclusion of sets of studies is given.

Each section of Volume 100 was reviewed by a subgroup of the Working Group with appropriate subject expertise; then all sections of each *Monograph* were discussed together in a plenary session of the full Working Group. As a result, the evaluation statements and other conclusions reflect the views of the Working Group as a whole.

Volume 100 compiles information on tumour sites and mechanisms of carcinogenesis. This information will be used in two scientific publications that may be considered as annexes to this volume. One publication, *Tumour Site Concordance between Humans and Experimental Animals*, will analyse the correspondence of tumour sites among humans and different animal species. It will discuss the predictive value of different animal tumours for cancer in humans, and perhaps identify human tumour sites for which there are no good animal models. Another publication, *Mechanisms Involved in Human Carcinogenesis*, will describe mechanisms known to or likely to cause cancer in humans. Joint consideration of multiple agents that act through similar mechanisms should facilitate the development of a more comprehensive discussion of these mechanisms. Because susceptibility often has its basis in a mechanism, this could also facilitate a more confident and precise description of populations that may be susceptible to agents acting through each mechanism. This publication will also suggest biomarkers that could render future research more informative. In this way, IARC hopes that Volume 100 will serve to improve the design of future cancer studies.

Specific remarks about the review of the agents in this volume

1. Arsenic and metals

One issue for several of these agents was the designation of the agent classified as carcinogenic. Arsenic and the metals considered exist in several oxidation states and in different forms that have different chemical and physical properties: metallic/elemental forms, alloys, and multiple compounds. For arsenic and the metals, the Working Group needed to consider whether:

1) the metallic/elemental form itself is carcinogenic;
2) the metallic/elemental form and the compounds are carcinogenic; or
3) only certain compounds are carcinogenic.

The simultaneous review of arsenic and multiple metals in this volume offered the opportunity for the Working Group to address the designation of these elements and/or their compounds in a uniform fashion. There had been some lack of consistency in prior designations, in part reflecting the nature of the evidence available and precedents in terminology around specific elements. Arsenic, for example, is widely referred to as "arsenic" alone and not as "arsenic and arsenic compounds."

In the *Monograph* on nickel and nickel compounds, the Working Group phrased its evaluation of the epidemiological studies as "mixtures of nickel compounds and nickel metal." The overall evaluation, however, was constrained to cover only nickel compounds and not nickel metal, in accordance with IARC's previously announced plan that Volume 100 would evaluate agents that had been classified as *carcinogenic to humans (Group 1)* in Volumes 1–99, and only nickel compounds had been classified in Group 1 in Volume 49 (IARC, 1990). Based on the previous evaluation in Volume 49, nickel metal remains classified as *possibly carcinogenic to humans (Group 2B).* The Working Group

recommends that there is a need for IARC to re-evaluate nickel metal in the near future in the context of the review of nickel compounds in this volume.

The situation was similar for chromium in that the review in Volume 100 considered the carcinogenicity of chromium (VI), but not of chromium with other oxidation states. The decision to omit metallic chromium or chromium (III) compounds from present assessment should not be interpreted as implying that these compounds are not carcinogenic or that the current evidence base is unchanged from that at the time of Volume 49 (IARC, 1990). Indeed, the evidence base has expanded and the Working Group does not pre-judge what the results of a new evaluation might be.

In the *Monograph* on arsenic and arsenic compounds, the Working Group developed a single updated assessment of agents that had been evaluated in previous *Monograph*s on arsenic and arsenic compounds (Volume 23 and Supplement 7, IARC, 1980, 1987a), arsenic in drinking-water (Volume 84, IARC, 2004), and gallium arsenide (Volume 86, IARC, 2006). It should be understood that arsenic in drinking-water and gallium arsenide should continue to be regarded as *carcinogenic to humans*, covered in this volume by the evaluation of arsenic and inorganic arsenic compounds.

In interpreting the human evidence on these agents, a particular difficulty was posed by the mixed exposures sustained by the worker populations included in the cohort studies. For groups exposed simultaneously to an agent in elemental/metallic form and to its compounds, the evidence may be uninformative as to the components of the mixture that cause cancer. When the evidence comes only from mixed exposure circumstances, the Working Group considered that the evaluation should be phrased as referring to "exposure to the element and its compounds."

This phrasing should not be interpreted as meaning that:

1) separate human evidence is available for the metallic/elemental form itself and for each of its compounds or

2) the evaluation of human evidence applies separately to the metallic/elemental form and to each of its compounds.

From the human evidence, insight can be gained as to the specific carcinogenic agent if sufficient informative studies are available on multiple cohorts having exposures to differing speciations of the element. Additionally, cancer bioassay and mechanistic evidence are critical to determining which components of the exposure mixture are carcinogenic, and were given full consideration by the Working Group.

2. Fibres and Dusts

When an agent is referred to as a dust, the assumption made by the Working Group was that the major route of exposure was by inhalation.

The assessment of toxicity and carcinogenicity of poorly soluble materials in the form of particles or fibres is difficult for the following reasons:

First, chemical composition alone does not fully define the relevant biological properties of particulate materials.

Second, particulate and fibrous carcinogens may undergo more complex metabolic transformation than other chemical agents. The surface of dusts may be modified *in vivo*, for example, there may be removal or deposition of metal ions or protein adsorption. These *in vivo* modifications may alter potency of the native particles or fibres.

Third, when comparing potency of dust particles, surface area may be a more appropriate dose metric than mass. In many cases, the extent of particle-derived free radicals and release of inflammatory mediators and the subsequent biological response correlate with surface area.

Fourth, particles and fibres with low solubility including quartz and asbestos fibres induce toxicity in the particulate form and not as individual molecules or ions. Particles and fibres may be deposited and retained in a focal area for a long time and contribute to the induction of lesions at this site. Particles and fibres may also be translocated to extrapulmonary sites.

Two occupations previously classified in Group 1 are considered in this volume. Boot and shoe manufacture and repair was previously evaluated in Volume 25 and in Supplement 7 (IARC, 1981, 1987a). In this volume, the Working Group concluded that the nasal sinus tumours and leukaemias observed in the epidemiological studies could be attributed to exposure to leather dust and to benzene, respectively. In accordance with the Preamble (see part B, Section 6a), the Working Group focused its evaluation more narrowly on leather dust, after searching for other studies involving this new agent. The Working Group renamed this *Monograph* "Leather Dust." (The *Monograph* on Benzene will be updated in Part F of Volume 100.)

Furniture and cabinet making was also previously evaluated in Volume 25 and in Supplement 7 (IARC, 1981, 1987a). In this volume, the Working Group concluded that the tumours of the nasal sinus and nasopharynx observed in the epidemiological studies could be attributed to exposure to wood dust or formaldehyde. Accordingly, these studies are reviewed in this volume in the *Monograph* on Wood Dust. (The *Monograph* on Formaldehyde will also be updated in Part F of Volume 100.)

The previous *IARC Monographs* on Talc Containing Asbestiform Fibres (Volume 42 and Supplement 7, IARC, 1987a, b) concerned talc described as containing asbestiform tremolite and anthophyllite. These fibres fit the definition of asbestos and therefore a separate review of talc containing asbestiform fibres was not undertaken. The studies on talc containing asbestiform fibres were considered when developing the *Monograph* on asbestos. Talc containing asbestos as well as other mixtures containing asbestos should be regarded as *carcinogenic to humans*.

In evaluating the carcinogenicity of asbestos fibres, the Working Group evaluated experimental data using the six types of asbestos fibres (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite and Anthophyllite) and erionite based on *in vitro* cellular assays and/or cancer bioassays. It should be understood that minerals containing asbestos in any form should be regarded as *carcinogenic to humans*. The Working Group agreed that the most important physicochemical properties of asbestos fibres relevant for toxicity and carcinogenicity are surface chemistry and reactivity, surface area, fibre dimensions, and biopersistence. Extrapolation of toxicity to other crystalline mineral fibres should not be done in the absence of epidemiological or experimental data based on *in vitro* and *in vivo* assays.

The toxicity of crystalline silica dusts obtained from different sources may be related to their geological history, process of particle formation, modifications during mining, processing and use, or surface contaminants even in trace amounts. Freshly ground crystalline silica exhibits a higher toxic potential than aged dusts. Crystalline silica may occur embedded in clays and other minerals or may be mixed with other materials in commercial products. It is possible that these other minerals or materials may adsorb onto the surface of crystalline silica dust and modify its reactivity. However, the extent of surface coverage and the potency of these modified dusts after residence in the lungs have not been systematically assessed.

3. Cross-cutting issues

3.1 Epidemiology

The epidemiological evidence considered in this Volume largely comes from studies of worker groups exposed to the agents under consideration. Additionally, population-based case–control studies also supply relevant evidence as do a few case series. There are several general issues related to these lines of epidemiological evidence that are covered in these comments.

The epidemiological evidence considered in this Volume largely comes from studies of worker groups exposed to the agents under consideration at levels that were high in relation to contemporary exposures, particularly in more developed countries. The cohort studies of workers have the general design of longitudinal follow-up of groups known to be exposed to the agent of interest in their workplace. Some cohort studies incorporate specific, unexposed comparison populations whereas others make a comparison to the rates of mortality in the general population, typically at the national level but sometimes on smaller geographic domains, e.g. states or counties. The measures of association used (e.g. standardized mortality ratios or SMRs) compare the rate of outcome in the exposed population to that in the unexposed population. One general concern in interpreting these measures of association is the appropriateness of the comparison population selected. National rates are often used because they are available and stable, but use of such rates may be inappropriate if there are important differences between the study population and the population at large on factors that might confound or modify the relationship between exposure and outcome. With appropriate consideration, local rates may be more suitable because factors that may confound the relationship between cancer risk and exposure, e.g. cigarette smoking, are likely to be more similar than a national population to the distributions in the worker population. Use of both national and local rates provides a sensitivity analysis as to the potential role of confounding. However, use of local rates may introduce bias if they are influenced by occupational or environmental exposures resulting from the plants under study, or if the geographical areas available for analyses do not reflect the areas from which the occupational population as drawn. Use of local rates may also result in imprecision of the epidemiological risk estimate due to instability resulting from small numbers and/or inaccuracies in small area data. The most appropriate comparison group would be other worker populations.

The informativeness of a cohort study depends on its size, i.e. the numbers of participants and outcome events. The sample sizes of the various cohort studies reflect the numbers of workers employed during the period of interest. Many of the studies had small population sizes, leading to imprecise measures of association, i.e. with wide confidence intervals. For some agents, small studies were set aside because they were uninformative. The Working Group did not attempt to combine the results of all studies, regardless of size, using quantitative meta-analysis.

3.2 Mixed exposures

In many of the cohorts studied, the workers were exposed to mixtures generated by industrial processes that contained not only the agent(s) of concern, but other potentially carcinogenic agents as well. For example, in some populations exposed to chromium, there was simultaneous exposure to arsenic. In analyses of the data from such studies, efforts were made to separate the effect of the agent of concern from the effects of other, potentially confounding agents. Such disentanglement is

possible only if the exposures are not highly correlated and the requisite data on exposures to the agents are available. There is also the assumption underlying such analyses that the effects of the various agents in the mixture are independent. In its deliberations, the Working Group recognized that exposures to many of the agents took place through exposures to mixtures containing them and took this into account in its interpretation of the evidence.

Exposures were estimated for study participants using approaches that typically were based on measurements and reconstruction of exposures based on work history and job–exposure matrices. Additionally, duration of employment was used as a surrogate for exposure. The measures of exposure were used in analyses directed at characterizing exposure–response relationships. Given the limited data available for estimating exposures, the exposure measures were subject to some degree of misclassification, likely random. One consequence of such exposure misclassification would be a blunting of estimated exposure–response relationships.

3.3 Smoking as confounder

In interpreting findings related to lung cancer and other sites for which smoking is a cause, there is the potential for confounding by smoking, particularly because many studies lacked information on smoking and direct adjustment for smoking was not possible. In assessing the potential for confounding by smoking, consideration was given to whether internal comparisons were made, which should not be as likely to be confounded as external comparisons. Additionally, some studies used available smoking information to estimate the potential for confounding by smoking. Such analyses are useful but have the underlying assumption that the effects of smoking and the agent of interest are independent.

Since the prior reviews, several data sets had undergone re-analysis by analysts who were not the original investigators. As appropriate, the Working Group considered these re-analyses to assess any insights into the original analyses.

A summary of the findings of this volume appears in The Lancet Oncology (Straif et al., 2009).

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ARSENIC AND ARSENIC COMPOUNDS

Arsenic and arsenic compounds were considered by previous IARC Working Groups in 1979, 1987, and 2002 (IARC, 1980, 1987, 2004). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

Information on the physical and chemical properties of arsenic and arsenic compounds can be found in Table 1.1, for further details please refer to IARC (1980). The list is not exhaustive, nor does it comprise necessarily the most commercially important arsenic-containing substances; rather, it indicates the range of arsenic compounds available.

1.2 Chemical and physical properties of the agents

Arsenic (atomic number, 33; relative atomic mass, 74.92) has chemical and physical properties intermediate between a metal and a non-metal, and is often referred to as a metalloid or semi-metal. It belongs to Group VA of the Periodic Table, and can exist in four oxidation states: −3, 0, +3, and +5. Arsenite, As^{III}, and arsenate, As^{V}, are the predominant oxidation states under, respectively, reducing and oxygenated conditions (WHO, 2001; IARC, 2004).

From a biological and toxicological perspective, there are three major groups of arsenic compounds:

-inorganic arsenic compounds,

-organic arsenic compounds, and

-arsine gas.

Of the inorganic arsenic compounds, arsenic trioxide, sodium arsenite and arsenic trichloride are the most common trivalent compounds, and arsenic pentoxide, arsenic acid and arsenates (e.g. lead arsenate and calcium arsenate) are the most common pentavalent compounds. Common organic arsenic compounds include arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid), and arsenobetaine (WHO, 2000).

1.3 Use of the agents

Arsenic and arsenic compounds have been produced and used commercially for centuries. Current and historical uses of arsenic include pharmaceuticals, wood preservatives, agricultural chemicals, and applications in the mining, metallurgical, glass-making, and semiconductor industries.

Arsenic was used in some medicinal applications until the 1970s. Inorganic arsenic was used

Table 1.1 Chemical names, CAS numbers, synonyms, and molecular formulae of arsenic and arsenic compounds

Chemical name	CAS Reg. No.	Synonyms	Formula
Arsanilic acid	98-50-0	Arsonic acid, (4-aminophenyl)-	$C_6H_8AsNO_3$
Arsenic[a]	7440-38-2	Metallic arsenic	As
Arsenic(V) pentoxide[b]	1303-28-2	Arsenic oxide [As_2O_5]	As_2O_5
Arsenic(III) sulfide	1303-33-9	Arsenic sulfide [As_2S_3]	As_2S_3
Arsenic(III) trichloride	7784-34-1	Arsenic chloride [$AsCl_3$]	$AsCl_3$
Arsenic(III) trioxide[a,c]	1327-53-3	Arsenic oxide [As_2O_3]	As_2O_3
Arsenobetaine	64436-13-1	Arsonium, (carboxymethyl) trimethyl-, hydroxide, inner salt; 2-(trimethylarsonio)acetate	$C_5H_{11}AsO_2$
Arsine	7784-42-1	Arsenic hydride	AsH_3
Calcium arsenate	7778-44-1	Arsenic acid [H_3AsO_4] calcium salt (2:3)	$(AsO_4)_2.3Ca$
Dimethylarsinic acid	75-60-5	Cacodylic acid	$C_2H_7AsO_2$
Lead arsenate	7784-40-9	Arsenic acid [H_3AsO_4], lead (2+) salt (1:1)	$HAsO_4.Pb$
Methanearsonic acid, disodium salt	144-21-8	Arsonic acid, methyl-, disodium salt	$CH_3AsO_3.2Na$
Methanearsonic acid, monosodium salt	2163-80-6	Arsonic acid, methyl-, monosodium salt	$CH_4AsO_3.Na$
Potassium arsenate[d]	7784-41-0	Arsenic acid [H_3AsO_4], monopotassium salt	$H_2AsO_4.K$
Potassium arsenite	13464-35-2	Arsenous acid, potassium salt	$AsO_2.K$
Sodium arsenate[e]	7631-89-2	Arsenic acid, [H_3AsO_4], monosodium salt	$H_2AsO_4.Na$
Sodium arsenite	7784-46-5	Arsenous acid, sodium salt	$AsO_2.Na$
Sodium cacodylate	124-65-2	Arsinic acid, dimethyl-, sodium salt	$C_2H_6AsO_2.Na$

[a] As_2O_3 is sometimes erroneously called 'arsenic'.
[b] The name 'arsenic acid' is commonly used for As_2O_5 as well as for the various hydrated products (H_3AsO_4, $H_4As_2O_7$).
[c] As_2O_3 is sometimes called 'arsenic oxide', but this name is more properly used for As_2O_5.
[d] The other salts, K_3AsO_4 and K_2HAsO_4, do not appear to be produced commercially.
[e] The name 'sodium arsenate' is also applied to both the disodium [7778-43-0] and the trisodium [13464-38-5] salts; it is therefore not always possible to determine which substance is under discussion.

in the treatment of leukaemia, psoriasis, and chronic bronchial asthma, and organic arsenic was used in antibiotics for the treatment of spirochetal and protozoal disease (ATSDR, 2007).

Inorganic arsenic is an active component of chromated copper arsenate, an antifungal wood preservative used to make "pressure-treated" wood for outdoor applications. Chromated copper arsenate is no longer used in residential applications, following a voluntary ban on its use in Canada and the United States of America at the end of 2003.

In the agricultural industry, arsenic has historically been used in a range of applications, including pesticides, herbicides, insecticides, cotton desiccants, defoliants, and soil sterilants. Inorganic arsenic pesticides have not been used for agricultural purposes in the USA since 1993. Organic forms of arsenic were constituents of some agricultural pesticides in the USA. However, in 2009, the US Environmental Protection Agency issued a cancellation order to eliminate and phase out the use of organic arsenical pesticides by 2013 (EPA, 2009). The one exception to the order is monosodium methanearsonate (MSMA), a broadleaf weed herbicide, which will continue to be approved for use on cotton. Small amounts of disodium methanearsonate (DSMA, or cacodylic acid) were historically applied to cotton fields as herbicides, but its use is now prohibited under the aforementioned US EPA 2009 organic arsenical product cancellation. Other organic

arsenicals (e.g. roxarsone, arsanilic acid and its derivatives) are used as feed additives for poultry and swine to increase the rate of weight gain, to improve feed efficiencies, pigmentation, and disease treatment and prevention (EPA, 2000, 2006; FDA, 2008a, b).

Arsenic and arsenic compounds are used for a variety of other industrial purposes. Elemental arsenic is used in the manufacture of alloys, particularly with lead (e.g. in lead acid batteries) and copper. Gallium arsenide and arsine are widely used in the semiconductor and electronics industries. Because of its high electron mobility, as well as light-emitting, electromagnetic and photovoltaic properties, gallium arsenide is used in high-speed semiconductor devices, high-power microwave and millimetre-wave devices, and opto-electronic devices, including fibre-optic sources and detectors (IARC, 2006). Arsine is used as a doping agent to manufacture crystals for computer chips and fibre optics.

Arsenic and arsenic compounds are used in the manufacture of pigments, sheep-dips, leather preservatives, and poisonous baits. They are also used in catalysts, pyrotechnics, antifouling agents in paints, pharmaceutical substances, dyes and soaps, ceramics, alloys (automotive solder and radiators), and electrophotography.

Historically, the USA has been the world's largest consumer of arsenic. Prior to 2004, about 90% of the arsenic consumed, as arsenic trioxide, was in the manufacture of wood preservatives. Since the voluntary ban on chromated copper arsenate in residential applications came into effect at the end of 2003, the consumption of arsenic for wood preservation has declined, dropping to 50% in 2007 (USGS, 2008). During 1990–2002, approximately 4% of arsenic produced was used in the manufacture of glass, and 1–4% was used in the production of non-ferrous alloys (NTP, 2005).

1.4 Environmental occurrence

Arsenic is the 20th most common element in the earth's crust, and is emitted to the environment as a result of volcanic activity and industrial activities. Mining, smelting of non-ferrous metals and burning of fossil fuels are the major anthropogenic sources of arsenic contamination of air, water, and soil (primarily in the form of arsenic trioxide). The historical use of arsenic-containing pesticides has left large tracts of agricultural land contaminated. The use of arsenic in the preservation of timber has also led to contamination of the environment (WHO, 2000, 2001).

1.4.1 Natural occurrence

In nature, arsenic occurs primarily in its sulfide form in complex minerals containing silver, lead, copper, nickel, antimony, cobalt, and iron. Arsenic is present in more than 200 mineral species, the most common of which is arsenopyrite. Terrestrial abundance of arsenic is approximately 5 mg/kg, although higher concentrations are associated with sulfide deposits. Sedimentary iron and manganese ores as well as phosphate-rock deposits occasionally contain levels of arsenic up to 2900 mg/kg (WHO, 2001).

1.4.2 Air

Arsenic is emitted to the atmosphere from both natural and anthropogenic sources. Approximately one-third of the global atmospheric flux of arsenic is estimated to be from natural sources (7900 tonnes per year). Volcanic activity is the most important natural contributor, followed by low-temperature volatilization, exudates from vegetation, and windblown dusts. Anthropogenic sources are estimated to account for nearly 24000 tonnes of arsenic emitted to the global atmosphere per year. These emissions arise from the mining and smelting of base metals, fuel combustion (e.g. waste and low-grade brown

coal), and the use of arsenic-based pesticides (WHO, 2000, 2001).

Arsenic is present in the air of suburban, urban, and industrial areas mainly as inorganic particulate (a variable mixture of As^{III} and As^{V}, with the pentavalent form predominating). Methylated arsenic is assumed to be a minor component of atmospheric arsenic (WHO, 2000). Mean total arsenic concentrations in air range from 0.02–4 ng/m^3 in remote and rural areas, and from 3–200 ng/m^3 in urban areas. Much higher concentrations (> 1000 ng/m^3) have been measured in the vicinity of industrial sources, such as non-ferrous metal smelters, and arsenic-rich coal-burning power plants (WHO, 2001).

1.4.3 Water

Arsenic, from both natural and anthropogenic sources, is mainly transported in the environment by water. The form and concentration of arsenic depends on several factors, including whether the water is oxygenated (for example, arsenites predominate under reducing conditions such as those found in deep well-waters), the degree of biological activity (which is associated with the conversion of inorganic arsenic to methylated arsenic acids), the type of water source (for example, open ocean seawater versus surface freshwater versus groundwater), and the proximity of the water source to arsenic-rich geological formations and other anthropogenic sources (WHO, 2000, 2001).

The concentration of arsenic in surface freshwater sources, like rivers and lakes, is typically less than 10 μg/L, although it can be as high as 5 mg/L near anthropogenic sources. Concentrations of arsenic in open ocean seawater and groundwater average 1–2 μg/L, although groundwater concentrations can be up to 3 mg/L in areas with volcanic rock and sulfide mineral deposits (WHO, 2001).

Exposure to high levels of arsenic in drinking-water has been recognized for many decades in some regions of the world, notably in the People's Republic of China, Taiwan (China), and some countries in Central and South America. More recently, several other regions have reported having drinking-water that is highly contaminated with arsenic. In most of these regions, the drinking-water source is groundwater, naturally contaminated from arsenic-rich geological formations. The primary regions where high concentrations of arsenic have been measured in drinking-water include large areas of Bangladesh, China, West Bengal (India), and smaller areas of Argentina, Australia, Chile, Mexico, Taiwan (China), the USA, and Viet Nam. In some areas of Japan, Mexico, Thailand, Brazil, Australia, and the USA, mining, smelting and other industrial activities have contributed to elevated concentrations of arsenic in local water sources (IARC, 2004).

Levels of arsenic in affected areas may range from tens to hundreds or even thousands of micrograms per litre, whereas in unaffected areas, levels are typically only a few micrograms per litre. Arsenic occurs in drinking-water primarily as As^{V}, although in reducing environments significant concentrations of As^{III} have also been reported. Trace amounts of methylated arsenic species are typically found in drinking-water, and higher levels are found in biological systems. More complete data on arsenic in water may be found in the previous *IARC Monograph* (IARC, 2004).

1.4.4 Soil and sediments

Natural and anthropogenic sources contribute to the levels of arsenic found in soil and sediments. Mean background concentrations in soil are often around 5 mg/kg, but can range from as low as 1 mg/kg to as high as 40 mg/kg. This variation in levels of naturally occurring arsenic in soils is associated with the presence of geological formations (e.g. sulfide ores, mineral sediments beneath peat bogs). Soils contaminated with arsenic from anthropogenic sources (e.g. mine/

smelter wastes, agricultural land treated with arsenical pesticides) can have concentrations of arsenic up to several grams per kilogram. Mean sediment arsenic concentrations range from 5–3000 mg/kg, with the higher levels occurring in areas of anthropogenic contamination (WHO, 2001).

1.5 Human exposure

1.5.1 Exposure of the general population

The primary route of arsenic exposure for the general population is via the ingestion of contaminated food or water. The daily intake of total arsenic from food and beverages is generally in the range of 20–300 μg/day.

Inhalation of arsenic from ambient air is generally a minor exposure route for the general population. Assuming a breathing rate of 20 m^3/day, the estimated daily intake may amount to about 20–200 ng in rural areas, 400–600 ng in cities without substantial industrial emission of arsenic, about 1 μg/day in a non-smoker and more in polluted areas, and up to approximately 10 μg/day in a smoker (WHO, 2000, 2001).

1.5.2 Occupational exposure

Inhalation of arsenic-containing particulates is the primary route of occupational exposure, but ingestion and dermal exposure may be significant in particular situations (e.g. during preparation of timber treated with chromated copper arsenate). Historically, the greatest occupational exposure to arsenic occurred in the smelting of non-ferrous metal, in which arseniferous ores are commonly used. Other industries or industrial activities where workers are or were exposed to arsenic include: coal-fired power plants, battery assembly, preparation of or work with pressure-treated wood, glass-manufacturing, and the electronics industry. Estimates of the number of workers potentially exposed to arsenic and arsenic compounds have been developed by the NIOSH in the USA and by CAREX in Europe. Based on the National Occupation Exposure Survey (NOES), conducted during 1981–83, NIOSH estimated that 70000 workers, including approximately 16000 female workers, were potentially exposed to arsenic and arsenic compounds in the workplace (NIOSH, 1990). Based on occupational exposure to known and suspected carcinogens collected during 1990–93, the CAREX (CARcinogen EXposure) database estimated that 147569 workers were exposed to arsenic and arsenic compounds in the European Union, with over 50% of workers employed in the non-ferrous base metal industries (n = 40426), manufacture of wood and wood and cork products except furniture (n = 33959), and construction (n = 14740). CAREX Canada estimates that 25000 Canadians are exposed to arsenic in their workplaces (CAREX Canada, 2011). These industries include: sawmills and wood preservation, construction, farms, non-ferrous metal (except aluminium) production and processing, iron and steel mills and ferro-alloy manufacturing, oil and gas extraction, metal ore mining, glass and glass-product manufacturing, semiconductor manufacturing, and basic chemical manufacturing.

1.5.3 Dietary exposure

Low levels of inorganic and organic arsenic have been measured in most foodstuffs (typical concentrations are less than 0.25 mg/kg). Factors influencing the total concentration of arsenic in food include: food type (e.g. seafood versus meat or dairy), growing conditions (e.g. soil type, water, use of arsenic-containing pesticides), and food-processing techniques. The highest concentrations of arsenic have been found in seafood (2.4–16.7 mg/kg in marine fish, 3.5 mg/kg in mussels, and more than 100 mg/kg in certain crustaceans), followed by meats, cereals, vegetables, fruit, and dairy products. Inorganic arsenic

is the predominant form found in meats, poultry, dairy products and cereal, and organic arsenic (e.g. arsenobetaine) predominates in seafood, fruit, and vegetables (WHO, 2000, 2001).

Regional differences are seen in the daily intake of total arsenic through food, and are mainly attributable to variations in the quantity of seafood consumed. For example, the daily dietary intake of total arsenic in Japan is higher than that in Europe and the USA (WHO, 2000). Based on the limited data available, it is estimated that approximately 25% of daily dietary arsenic intake is from inorganic sources. Arsenic intake is typically higher in men than it is in women and children, with estimated levels ranging from 1.3 µg/day for infants under 1 year of age, 4.4 µg/day for 2-year olds, 9.9 µg/day for 25–30-year-old men, 10 µg/day for 60–65-year-old women, and 13 µg/day for 60–65-year-old men (WHO, 2001).

1.5.4 Biomarkers of exposure

Arsine generation atomic absorption spectrometry (AAS) is the method of choice for biological monitoring of exposure to inorganic arsenic (WHO, 2000). The absorbed dose of arsenic can be identified and quantified in hair, nail, blood or urine samples. Because arsenic accumulates in keratin-rich tissue, total arsenic levels in hair, fingernails or toenails are used as indicators of past exposures. In contrast, because of its rapid clearing and metabolism, blood arsenic, urine arsenic, and urine arsenic metabolites (inorganic arsenic, monomethylarsonic acid [MMA^V] and dimethylarsinic acid [DMA^V]) are typically used as indicators of recent exposure.

The concentration of metabolites of inorganic arsenic in urine generally ranges from 5–20 µg/L, but may exceed 1000 µg/L (WHO, 2001). Time-weighted average (TWA) occupational exposure to airborne arsenic trioxide is significantly correlated with the inorganic arsenic metabolites in urine collected immediately after a shift or just before the next shift. For example, at an airborne concentration of 50 µg/m^3, the mean concentration of arsenic derived from the sum of the three inorganic arsenic metabolites in a post-shift urine sample was 55 µg/g of creatinine. In non-occupationally exposed subjects, the sum of the concentration of the three metabolites in urine is usually less than 10 µg/g of creatinine (WHO, 2000).

2. Cancer in Humans

The epidemiological evidence on arsenic and cancer risk comes from two distinct lines of population studies, characterized by the medium of exposure to arsenic. One set of studies addresses the cancer risk associated with inhalation. These studies involve populations that are largely worker groups who inhaled air contaminated by arsenic and other agents, as a consequence of various industrial processes. The second set of studies was carried out in locations where people ingested arsenic in drinking-water at high concentrations over prolonged periods of time.

2.1 Types of human exposure circumstances studied

2.1.1 Arsenic exposure by inhalation

The cohort studies and nested case–control studies considered in this *Monograph* that are relevant to airborne arsenic include workers in metal smelters and refineries, and miners of various ores. Case–control studies within the general population addressed occupational exposures more generally. Consequently, the exposure to inhaled arsenic was accompanied by exposures to other potentially toxic and carcinogenic by-products of combustion, such as sulfur oxides with copper smelting, polycyclic aromatic hydrocarbons, and particulate matter.

Most studies did not attempt to estimate separately exposures to the full set of agents in the inhaled mixtures, leaving open the possibility of some confounding or modification of the effect of arsenic by synergistic interactions.

2.1.2 Arsenic exposure by ingestion

For most human carcinogens, the major source of evidence contributing to causal inferences arises from case–control and cohort studies. In contrast, for arsenic in drinking-water, ecological studies provide important information on causal inference, because of the large exposure contrasts and the limited population migration. For arsenic, ecological estimates of relative risk are often so high that potential confounding with known causal factors could not explain the results. Although food may also be a source of some ingested arsenic, in several regions of the world where the concentrations of arsenic in drinking-water is very high, arsenic intake through food consumption contributes a relatively small cancer risk to the local residents (Liu *et al.*, 2006a).

The strongest evidence for the association of human cancer with arsenic in drinking-water comes from studies in five areas of the world with especially elevated levels of naturally occurring arsenic: south-western and north-eastern Taiwan (China), northern Chile, Cordoba Province in Argentina, Bangladesh, West Bengal (India), and other regions in the Ganga plain. Although data contributing to our understanding also come from many other places, the current review is largely restricted to the major studies from these regions. Some of the oral exposure may occur via seafood. However, no epidemiological studies were available with regard to the cancer risk associated with arsenic exposure via seafood, in which arsenic may occur as particular organic compounds such as arsenobetaine and arsenocholine.

(a) Taiwan (China)

Exposure to arsenic was endemic in two areas of Taiwan (China): The south-western coastal area (Chen *et al.*, 1985), and the north-eastern Lanyang Basin (Chiou *et al.*, 2001). Residents in the south-western areas drank artesian well-water with high concentrations of arsenic from the early 1910s to the late 1970s, with levels mostly above 100 µg/L (Kuo, 1968; Tseng *et al.*, 1968). In the Lanyang Basin, residents used arsenic-contaminated water from household tube wells starting in the late 1940s. Arsenic in the water of 3901 wells, tested in 1991–94 ranged from undetectable (< 0.15 µg/L) to 3.59 mg/L (median = 27.3 µg/L) (Chiou *et al.*, 2001).

(b) Northern Chile

The population-weighted average concentration of arsenic in drinking-water in Region II, an arid region of northern Chile, was about 570 µg/L over 15 years (1955–69) (Smith *et al.*, 1998). With the introduction of a water-treatment plant in 1970, levels decreased. By the late 1980s, arsenic levels in drinking-water had decreased to less than 100 µg/L in most places. With minor exceptions, water sources elsewhere in Chile have had low concentrations of arsenic (less than 10 µg/L) (Marshall *et al.*, 2007).

(c) Cordoba Province, Argentina

Of the 24 counties in Cordoba Province, two have been characterized as having elevated exposure to arsenic in drinking-water (average level, 178 µg/L), six as having medium exposure, and the remaining 16 rural counties as having low exposure (Hopenhayn-Rich *et al.*, 1996, 1998).

(d) Bangladesh, West Bengal (India), and other locations in the Ganga plain

Millions of tube wells were installed in West Bengal (India), Bangladesh, and other regions in the Ganga plain of India and Nepal starting in the late 1970s to prevent morbidity and mortality

from gastrointestinal disease (Smith *et al.*, 2000). Elevated arsenic in wells in Bangladesh was confirmed in 1993 (Khan *et al.*, 1997). In a Bangladesh survey by the British Geological Survey of 2022 water samples in 41 districts, 35% were found to have arsenic levels above 50 μg/L, and 8.4% were above 300 μg/L, with an estimate of about 21 million persons exposed to arsenic concentrations above 50 μg/L (Smith *et al.*, 2000).

2.2 Cancer of the lung

2.2.1 Exposure via inhalation

Several ecological studies were conducted on populations exposed to arsenic through industrial emissions. The worker studies primarily provide information on lung cancer. The case–control studies are also mostly directed at lung cancer, with one on non-melanoma skin cancer (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.1.pdf).

The cohort studies reviewed previously and here consistently show elevated lung cancer risk in the various arsenic-exposed cohorts compared with the general population or other comparison groups, with most values in the range of 2–3 (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.2.pdf and Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.3.pdf).

The studies incorporate diverse qualitative and quantitative indices of exposure that include measures of average airborne concentration of exposure, cumulative exposure across the work experience, and duration of exposure. There is consistent evidence for a positive exposure–response relationship between the indicators of exposure and lung cancer risk. Case–control studies nested within occupational cohorts provided similar evidence with regard to exposure–response relationships.

Several analyses further explored the relationship between arsenic exposure and lung cancer risk using models based on either empirical, i.e. descriptive, or biological data (see Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.4.pdf).

Using data from the Tacoma, Washington smelter workers, Enterline *et al.* (1987) modelled the relationship between lung cancer risk and airborne arsenic exposure using power functions, and found that the exposure–response relationship was steeper at lower concentrations than shown in conventional analyses, and was concave downwards at higher concentrations. By contrast, the relationship of risk with urine arsenic concentration was linear. Lubin *et al.* (2000, 2008) analysed the exposure–response relationship of lung cancer risk with arsenic exposure in the cohort of Montana smelter workers, now followed for over 50 years. Overall, a linear relationship of risk with cumulative exposure was found; however, the slope of the relationship increased with the average concentration at which exposure had taken place, that is, the effect of a particular cumulative exposure was greater if received at a faster rate.

For a comparison of the different studies, see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.5.pdf.

2.2.2 Exposure via ingestion

A summary of the findings of epidemiological studies on arsenic in drinking-water and risk for lung cancer are shown in Table 2.6 (water exposures) available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.6.pdf, and online Tables 2.1 to 2.4 (air exposures).

(a) Ecological studies

Ecological studies, based on mortality records, were conducted in the arseniasis endemic area of south-western Taiwan (China) (Chen *et al.*, 1985, 1988a; Wu *et al.*, 1989; Chen & Wang, 1990; Tsai *et al.*, 1999). All studies found elevated risks for lung cancer mortality associated with levels of arsenic in drinking-water, or surrogate measurements.

In Chile, Rivara *et al.* (1997) found an elevated relative risk (RR) for mortality from lung cancer in 1976–92 in Region II compared with Region VIII, a low-exposure area. Smith *et al.* (1998) found an elevated standardized mortality ratio (SMR) of approximately 3 for lung cancer for both sexes in Region II, using the national rate as standard. In Cordoba Province, Argentina, significant increases in lung cancer mortality were associated with increasing exposure to arsenic (Hopenhayn-Rich *et al.*, 1998). Smith *et al.* (2006) found an elevated lung cancer mortality (RR, 7.0; 95%CI: 5.4–8.9) among the 30–49-year-old residents of Antofagasta and Mejillones born in the period 1950–57, just before the period of exposure to high arsenic levels (1958–70). They were exposed in early childhood to high levels of arsenic through the drinking-water. The temporal pattern of lung cancer mortality rate ratios in Region II compared with that in Region V (a low-exposure area) from 1950 to 2000, showed an increase about 10 years after the onset of high arsenic exposure, and peaked in 1986–87, with relative risks of 3.61 (95%CI: 3.13–4.16) and 3.26 (95%CI: 2.50–4.23) for men and women, respectively (Marshall *et al.*, 2007).

(b) Case–control and cohort studies

In northern Chile, a case–control study of 151 cases and 419 controls reported significantly increasing risks with increasing levels of arsenic during the 1958–70 high-exposure period, with an odds ratio increasing to 7.1 (95%CI: 3.4–14.8) (Ferreccio *et al.*, 2000).

In a cohort from south-western Taiwan (China), Chen *et al.* (1986) observed a dose–response relationship between the duration of consumption of artesian well-water containing high levels of arsenic and lung cancer mortality risk, showing the highest age- and gender-adjusted odds ratio among those who consumed artesian well-water for more than 40 years compared with those who never consumed artesian well-water. Another cohort study from south-western Taiwan (China) endemic for arsenic found a smoking-adjusted increased risk for lung cancer in relation to increasing average concentrations of arsenic and increasing cumulative exposure to arsenic (Chiou *et al.*, 1995).

A further study of combined cohorts in south-western (n = 2503) and north-eastern (n = 8088) Taiwan (China) found a synergistic interaction between arsenic in drinking-water and cigarette smoking (Chen *et al.*, 2004).

A case–control study from Bangladesh, conducted in 2003–06, found an elevated risk (odds ratio [OR], 1.65; 95%CI: 1.25–2.18) for male smokers consuming tube well-water with arsenic levels of 101–400 µg/L (Mostafa *et al.*, 2008). In non-smokers, the study did not report an increased risk with increasing arsenic exposure. [The Working Group noted the ecological nature of the exposure estimates, the possibility of greater sensitivity to arsenic exposure among smokers, and the relatively short latent period, with almost two-thirds of the wells put in place in 1990 or later.]

2.3 Cancer of the urinary bladder and of the kidney

The results of the epidemiological studies on arsenic in drinking-water and the risk for cancers of the urinary bladder and of the kidney are summarized in Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.7.pdf.

2.3.1 Ecological studies

In south-western and north-eastern Taiwan (China), the relation between cancer of the urinary bladder and of the kidney and drinking-water containing arsenic was evaluated in many of the studies cited above (Chen *et al.*, 1985, 1988a; Wu *et al.*, 1989; Chen & Wang, 1990; Tsai *et al.*, 1999). Each reported an elevation in mortality from these cancers during various time periods in 1971–94 associated with levels of arsenic in well-water from rural artesian wells, with many reporting a dose–response relationship among both men and women. An additional study, based on incidence records, found comparable risks for bladder cancer (Chiang *et al.*, 1993).

In Region II of Chile, two studies found markedly high SMRs for cancer of the urinary bladder and of the kidney in 1950–92 (Rivara *et al.*, 1997) and in 1989–93 (Smith *et al.*, 1998). In the latter study, mortality from chronic obstructive pulmonary disease was at the expected level, suggesting that smoking was not involved. The temporal pattern of bladder cancer mortality in Region II from 1950–2000 was compared with that in Region V (Marshall *et al.*, 2007). Increased relative risks were reported about 10 years after the start of exposure to high arsenic levels, with peak relative risks of 6.10 (95%CI: 3.97–9.39) for men, and 13.8 (95%CI: 7.74–24.5) for women in the period 1986–94. In Cordoba Province, Argentina, positive trends in SMRs were reported for bladder and kidney cancers associated with estimates of exposure to arsenic in drinking-water (Hopenhayn-Rich *et al.*, 1996, 1998), again with no findings for chronic obstructive pulmonary disease.

[The Working Group noted that kidney cancers consist of both renal cell carcinoma and transitional cell carcinoma of the renal pelvis, the latter often being of the same etiology as bladder cancer. As arsenic causes transitional cell carcinoma of the bladder, merging of the two types of kidney cancer may result in a dilution of the risk estimate for total kidney cancer.]

2.3.2 Case–control and cohort studies

In a case–control study using death certificates (1980–82) from the area in Taiwan (China), endemic for Blackfoot disease, Chen *et al.* (1986) reported increasing trends in odds ratios with increasing duration of consumption of artesian well-water containing arsenic. The highest risks were seen for over 40 years of exposure, with an odds ratio of 4.1 ($P < 0.01$) for bladder cancer in a multivariate analysis, after adjusting for smoking and other factors from next-of-kin interviews.

In case–control studies of incident bladder cancer that included analysis of arsenic species in urine samples, a higher risk associated with arsenic was found among persons with higher MMA^V:DMA^V ratios or, alternatively, with a higher percentage of MMA^V (Chen *et al.*, 2003, 2005a; Steinmaus *et al.*, 2006; Pu *et al.*, 2007a; Huang *et al.*, 2008).

Cohort studies from south-western and north-eastern Taiwan (China) (Chen *et al.*, 1988b; Chiou *et al.*, 1995, 2001; Chen & Chiou, 2001) Japan (Tsuda *et al.*, 1995), and the United Kingdom (Cuzick *et al.*, 1992) each observed elevated bladder cancer risk following long-term exposure to ingested arsenic, with dose–response relationships found where the numbers of cases permitted such an analysis. The study from Taiwan (China), also found an elevated risk of kidney cancer (OR, 2.8; 95%CI: 1.3–5.4, based on nine cases) (Chiou *et al.*, 2001).

2.4 Cancer of the skin

The recognition of arsenic as a carcinogen first came from case series describing skin cancers following the ingestion of medicines containing arsenicals (Hutchinson, 1888; Neubauer, 1947), and exposure to arsenical pesticide residues, and arsenic-contaminated wine (Roth, 1957; Grobe,

1977) or drinking-water, originating from many countries. The characteristic arsenic-associated skin tumours include squamous cell carcinomas arising in keratoses (including Bowen disease), and multiple basal cell carcinomas.

Findings of epidemiological studies on arsenic in drinking-water and risk for skin cancer are summarized in Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.8.pdf.

2.4.1 Ecological studies of prevalence

In south-western Taiwan (China), Tseng *et al.* (1968) found an 8-fold difference in the prevalence of skin cancer lesions from the highest (> 600 µg/L) to the lowest category (< 300 µg/L) of arsenic concentration in artesian wells, after an extensive examination survey of 40421 inhabitants in 37 villages.

2.4.2 Ecological studies based on mortality from cancer of the skin

Studies in Taiwan (China) (Chen *et al.*, 1985, 1988a; Wu *et al.*, 1989; Chen & Wang, 1990; Tsai *et al.*, 1999) analysed skin cancer mortality in relation to levels of arsenic in well-water. These investigations found consistent gradients of increasing risk with average level of arsenic in drinking-water, as measured on the township or precinct level.

Rivara *et al.* (1997) observed an SMR for skin cancer of 3.2 (95%CI: 2.1–4.8), comparing mortality from skin cancer in 1976–92 between Region II and the unexposed control Region VIII of Chile. Later, Smith *et al.* (1998) found SMRs of 7.7 (95%CI: 4.7–11.9) among men and 3.2 (95%CI: 1.3–6.6) among women for the years 1989–93 in Region II of Chile, using national mortality rates as reference. [The Working Group noted that the histological type of skin cancer was reported in only a few instances. Although skin cancer mortality can be influenced by access to health care, the SMRs reported here are so large as to not be explained by any possible confounding.]

2.4.3 Cohort studies

A retrospective cohort study of 789 (437 men, 352 women) of Blackfoot disease patients in Taiwan (China) reported an SMR of 28 (95%CI: 11–59) for skin cancer deaths (based on seven observed deaths), using Taiwan (China) regional rates as reference (Chen *et al.*, 1988b).

In a cohort of 654 persons in south-western Taiwan (China), an observed incidence rate of 14.7 cases of skin cancer/1000 person–years was found (Hsueh *et al.*, 1997), with risks significantly related to duration of living in the area endemic for Blackfoot disease, duration of consumption of artesian well-water, average concentration of arsenic, and index for cumulative exposure to arsenic. Similar findings were observed in a nested case–control study conducted within this cohort (Hsueh *et al.*, 1995).

In Region II of Chile, a decrease in incidence rates of cutaneous lesions (leukoderma, melanoderma, hyperkeratosis, and squamous cell carcinoma) was observed during 1968–71 after a lowering of waterborne arsenic levels from a filter plant, which started operation in 1970 (Zaldívar, 1974).

2.5 Cancer of the liver

2.5.1 Ecological studies

The relation between liver cancer risk and drinking-water contaminated with arsenic was evaluated in many of the studies from south-western Taiwan (China), cited above (Chen *et al.*, 1985, 1988a; Wu *et al.*, 1989; Chen & Wang, 1990; Chiang *et al.*, 1993; Tsai *et al.*, 1999; see Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.9.pdf), with positive associations found in all studies.

In northern Chile, Rivara *et al.* (1997) observed a relative risk for liver cancer mortality of 1.2 (95%CI: 0.99–1.6) in arsenic-exposed Region II compared with Region VIII. Liver cancer mortality in Region II of northern Chile during the period 1989–93 among persons ≥ 30 years of age was not significantly elevated, using national rates as reference (Smith *et al.*, 1998). SMRs were 1.1 (95%CI: 0.8–1.5) both for men and for women. Liaw *et al.* (2008) found an elevated relative risk (10.6; 95%CI: 2.9–39.3, $P < 0.001$) for liver cancer among children in Region II of Chile born in 1950–57 and exposed *in utero* or shortly thereafter, compared to rates in Region V of Chile.

In Cordoba Province, Argentina, SMRs were not related to arsenic exposure (Hopenhayn-Rich *et al.*, 1998).

[The Working Group noted that the finding of an association with liver cancer in Taiwan (China), but not in South America may reflect a more sensitive population in the former region, due to endemic hepatitis B. The elevated risk of those exposed *in utero* and as young children may reflect a combination of greater biological vulnerability in early life (Waalkes *et al.*, 2007) plus the fact that young children consume 5–7 times more water per kilogram body weight per day than adults (NRC, 1993).]

2.5.2 Case–control studies

In a case–control study investigating the consumption artesian well-water containing high concentrations of arsenic and mortality from liver cancer in four townships of south-westernern Taiwan (China), Chen *et al.* (1986) observed an exposure–response relationship between the duration of consumption of the contaminated well-water and risk for liver cancer, adjusted for cigarette smoking, habitual alcohol and tea drinking, and consumption of vegetables and fermented beans.

2.6 Cancer of the prostate

Studies conducted in Taiwan (China) (Chen *et al.*, 1985, 1988a; Wu *et al.*, 1989; Chen & Wang, 1990; Tsai *et al.*, 1999) analysed prostate cancer mortality in relation to levels of arsenic in well-water, with some overlap among the respective study populations. Using several methodological approaches and comparison populations including direct and indirect standardization of rates, all studies reported significant dose–response relationships between the level of arsenic in drinking-water and the risk for prostate cancer mortality (see Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100C/100C-01-Table2.10.pdf).

In Chile, Rivara *et al.* (1997) found a relative risk of 0.9 (95%CI: 0.54–1.53) for prostate cancer, comparing the 1990 mortality rate for prostate cancer of Region II with that of Region VIII.

2.7 Synthesis

The Working Group reviewed a large body of evidence that covers ecological studies, case–control studies and cohort studies in a variety of settings and populations exposed either by ingestion (primarily to As^{III} and As^{V} in drinking-water) or inhalation (with exposure to a mixture of inorganic arsenic compounds). The evidence also relates to historical exposure from pesticidal and pharmaceutical uses. The epidemiological evidence from drinking-water exposure permits the evaluation of the carcinogenicity that is related to exposure to As^{III} and As^{V}. The epidemiological evidence from inhaled arsenic mixtures permits the evaluation of the carcinogenicity that is related to inorganic arsenic compounds. However, it does not allow a separation of the carcinogenic risk associated with particular arsenic species that occur in these mixtures.

The observed associations between exposure to arsenic in drinking-water and lung cancer, and between exposure to arsenic in air and lung

cancer, cannot be attributed to chance or bias. The evidence is compelling for both the inhalation and ingestion routes of exposure. There is evidence of dose–response relationships within exposed populations with both types of exposure.

The observed association between exposure to arsenic in drinking-water and bladder cancer cannot be attributed to chance or bias. There is evidence of dose–response relationships within exposed populations.

The observed association between exposure to arsenic in drinking-water and skin cancer cannot be attributed to chance or bias. There is evidence of dose–response relationships within exposed populations. The evidence is primarily for squamous cell carcinoma of the skin.

Although the data for kidney cancer are suggestive of a relationship with exposure to arsenic in drinking-water, overall, the small possibility of chance or bias cannot be completely ruled out.

The evidence for an association between liver cancer and long-term exposure to arsenic in drinking-water relies on mortality data. Although the data strongly suggest a causal association with some evidence of a dose–response relationship, the Working Group could not rule out possible chance or bias. The evidence comes mainly from Taiwan (China) where hepatitis B is highly prevalent.

The evidence for an association for prostate cancer and long-term exposure to arsenic in drinking-water relies on mortality data. In the studies from Taiwan (China), there is some evidence of a dose–response relationship. However, the data from South America are not consistent with this observation. Although the evidence on prostate cancer suggests the possibility of a causal association, the Working Group could not rule out the possibility of chance or bias.

3. Cancer in Experimental Animals

Over the years, it has proved difficult to provide evidence for the carcinogenesis of inorganic arsenic compounds. More recent work has focused on methylated arsenic metabolites in humans or exposure to inorganic arsenic during early life, and has provided the information to show potential links between arsenic and carcinogenesis.

Studies published since the previous *IARC Monograph* (IARC, 2004) are summarized below.

3.1 Oral administration

3.1.1 Mouse

The oral administration of sodium arsenate in drinking-water for 18 months increased lung tumour multiplicity and lung tumour size in male strain A/J mice (Cui *et al.*, 2006; see Table 3.1).

Similarly, drinking-water exposure to the organo-arsenical DMA^V for 50 weeks or more increased the incidence and multiplicity of lung adenoma or carcinoma in strain A/J mice (Hayashi *et al.*, 1998), and increased lung tumours in mutant *Ogg*−/− mice (which cannot repair certain types of oxidative DNA damage) but not in *Ogg*+/+ mice (Kinoshita *et al.*, 2007; see Table 3.2).

3.1.2 Rat

In male F344 rats, the oral administration of DMA^V in drinking-water for up to 2 years produced clear dose–response relationships for the induction of urinary bladder transitional cell carcinoma and combined papilloma or carcinoma (Wei *et al.*, 1999, 2002).

When DMA^V was added to the feed of male and female F344 rats for 2 years, a clear dose–response relationship for urinary bladder benign and/or malignant transitional cell tumours

Table 3.1 Studies of cancer in experimental animals exposed to sodium arsenate (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, A/J (M) 18 mo Cui *et al.* (2006)	0, 1, 10, 100 ppm arsenate in drinking-water, *ad libitum* 30/group	Lung (adenomas): 0/19, 0/13, 0/15, 4/30 (13%)	[NS, (any dose)][a]	Age at start, 5 wk Purity, NR Redundant Student *t*-test used for multiple comparisons of lung tumour multiplicity and size Survival significantly increased at high dose Non-dose-related, modest changes in bw, lung weight, and lung bw ratio
		Lung (adenocarcinomas): 9/19 (47%), 10/13 (77%), 11/15 (73%), 19/30 (63%)	[NS, (any dose)][a]	
		Average tumours/mouse lung: 0.59, 1.1, 1.3, 1.4[b]	$P < 0.01$ (all doses)	
		Average number tumours > 4 mm/mouse lung: 17, 32, 44, 60[b]	$P < 0.01$ (all doses)	

[a] Performed during review. One-sided Fisher Exact test–control versus all treated.
[b] Numbers are estimates at review because data are presented graphically in original work.
bw, body weight; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

occurred in female but not male rats (Arnold *et al.*, 2006). Preneoplasia (urothelial cell hyperplasia) was clearly increased in female rats (Arnold *et al.*, 2006; see Table 3.2).

In male F344 rats, the oral administration of trimethylarsine oxide in drinking-water for 2 years caused a significant increase of benign liver tumours (adenoma) (Shen *et al.*, 2007; see Table 3.3).

Oral exposure to MMA^{V} for 2 years was negative in a comprehensive dose–response study including male and female rats and mice, although body weight suppression and reduced survival with the higher doses confounded the rat segment of the study (Arnold *et al.*, 2003; see Table 3.4).

A 2-year dose–response study with sodium arsenite showed some evidence of renal tumour formation in female Sprague-Dawley rats but not in males (Soffritti *et al.*, 2006). Tumour incidence did not reach significance (see Table 3.5).

3.2 Intratracheal administration

3.2.1 Hamster

Repeated weekly intratracheal instillations of calcium arsenate, at levels sufficient to caused moderate early mortality, increased lung adenoma formation in male Syrian golden hamsters when observed over their lifespan (Pershagen & Björklund, 1985).

In a similarly designed study, male hamsters received multiple weekly intratracheal instillations of calcium arsenate at the start of the experiment, and developed an increased incidence of lung adenoma formation, and combined lung adenoma or carcinoma formation over their lifespan (Yamamoto *et al.*, 1987; see Table 3.6).

Intratracheal instillations of calcium arsenite increased the incidence of respiratory tract carcinoma and combined adenoma, papilloma and adenomatoid lesion formation in male Syrian Hamsters (Pershagen *et al.*, 1984; see Table 3.7).

Table 3.2 Studies of cancer in experimental animals exposed to dimethylarsinic acid, DMA^V (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, A/J (M) 50 wk Hayashi *et al.* (1998)	0, 50, 200, 400 ppm DMA^V in drinking-water, *ad libitum* 24/group	Number of mice with lung papillary adenomas or adenocarcinomas: 2/14 (14%), 5/14 (36%), 7/14 (50%), 10/13 (77%)	$P < 0.01$ (high dose)	Age at start, 5 wk Purity, NR Survival unremarkable [Only histologically confirmed tumours were considered by the Working Group]
Mouse, *Ogg1*-/- and *Ogg1*+/+ (M, F) 72 wk Kinoshita *et al.* (2007)	0, 200 ppm DMA^V in drinking-water, *ad libitum*; controls received tap water 10/group (*Ogg1*-/-) 12/group (*Ogg1*+/+)	***Ogg1-/-:*** Tumour-bearing mice (any site): 0/10, 10/10 (100%)	$P < 0.01$	Age at start, 14 wk Purity, 99% Bw and food and water consumption unremarkable Left lobe and visible lung nodules used for histopathological tumour analysis Treated *Ogg1*−/− showed modest decreased survival (~20%) late compared to phenotypic control Small groups
		Lung lesions– Hyperplasias: 10/10 (100%), 10/10 (100%)		
		Adenomas: 0/10, 2/10 (20%)	NS	
		Adenocarcinomas: 0/10, 3/10 (30%)		
		Total lung tumours: 0/10, 5/10 (50%)	$P < 0.05$	
		Tumours/mouse: 0, 0.5	$P < 0.05$	
		Ogg1+/+: Tumour-bearing mice (any site): 5/10 (50%), 6/10 (60%)		
		Lung lesions– Hyperplasias: 2/10 (20%), 10/10 (100%)	[$P < 0.01$[b]]	
		Adenomas: 1/10 (10%), 0/10	NS	
		Adenocarcinomas: 0/10, 0/10	NS	
		Total tumours: 1/10 (10%), 0/10	NS	
		Tumours/mouse: 0.1, 0	NS	
		Tumours/mouse: 0.1, 0	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 104 wk Wei *et al.* (1999)[d], 2002)	0, 12.5, 50, 200 ppm DMA^V in drinking-water, *ad libitum* 36/group	Urinary bladder (hyperplasias): 0/28, 0/33, 12/31 (39%), 14/31 (45%) Urinary bladder (papillomas): 0/28, 0/33, 2/31 (2%), 2/31 (2%) Urinary bladder (carcinomas): 0/28, 0/33, 6/31 (19%), 12/31 (39%) Urinary bladder (papillomas or carcinomas): 0/28, 0/33, 8/31 (26%), 12/31 (39%)	$P < 0.01$ (middle and high dose) NS $P < 0.05$ (middle dose) $P < 0.01$ (high dose) $P < 0.01$ (middle and high dose)	Age at start, 10 wk Purity, 99% Survival and food intake unaltered Transient bw suppression early with high and middle dose but then similar to control Water intake increased at highest two doses Incidence rates based on rats at risk (surviving to time of the first bladder tumour at 97 wk) Extensive necropsy
Rat, F344 (M, F) 104 wk Arnold *et al.* (2006)	0, 2, 10, 40, 100 ppm DMA^V in feed, *ad libitum* 60/group	**Females** Urothelial cell (hyperplasias, simple, nodular and papillary): 0/60, 1/59 (2%), 0/60, 29/59 (49%), 48/60 (80%)	$P < 0.01$ (trend) [$P < 0.01$ (highest, and second highest dose)][b]	Purity > 99%; age, 5 wk Complete necropsies performed No treatment-related differences in mortality or bw
		Urinary bladder (papillomas): 0/60, 0/59, 0/60, 0/59, 4/60 (7%)		Sporadic changes in food consumption not treatment-related
		Urinary bladder (carcinomas): 0/60, 0/59, 0/60, 0/59, 6/60 (10%)	[NS (high dose)][b] $P < 0.01$ (trend)[c]	Water consumption increased with treatment
		Urinary bladder (papillomas and carcinomas combined): 0/60, 0/59, 0/60, 0/59, 10/60 (3%)	[$P < 0.05$ (high dose)][b] $P < 0.01$ (trend)[c] [$P < 0.05$ (high dose)][b]	
		Males Urothelial cell (hyperplasias, simple, nodular and papillary): 0/60, 0/59, 0/60, 6/58 (10%), 40/59 (68%)	$P < 0.01$ (trend) [$P < 0.01$ (high dose)][b]	Water consumption increased with treatment
		Urinary bladder (papillomas): 0/60, 0/59, 1/60 (2%), 1/58 (2%), 0/59	[NS (high dose)][b] $P < 0.01$ (trend)[c]	
		Urinary bladder (carcinomas): 0/60, 1/59 (2%), 0/60, 0/58, 2/59 (3%)	[NS (high dose)][b] $P < 0.01$ (trend)[c]	
		Urinary bladder (papillomas and carcinomas combined): 0/60, 1/59 (2%), 1/60 (2%), 1/58 (2%), 2/59 (3%)	[NS (high dose)][b]	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (F) 104 wk Arnold *et al.* (2006)	0, 8, 40, 200, 500 ppm DMA^V in feed, *ad libitum* 56/group	**Females** No treatment-related changes in urinary bladder preneoplasia or tumour incidence noted Any organ (fibrosarcomas): 3/56 (5%), 0/55, 1/56 (2%), 1/56 (2%), 6/56 (11%) **Males** No treatment-related changes in urinary bladder preneoplasia or tumour incidence noted	 $P < 0.01$ (high dose)	Age at start, 5 wk Purity 99% Complete necropsies performed Survival, bw and water consumption unchanged Sporadic, small changes in food consumption early Fibrosarcomas not considered related to treatment by authors Bw reduced at 500 ppm throughout study

[a] Data also included descriptive statistics (i.e. SD).
[b] Performed during review. One-sided Fisher exact test control versus treated.
[c] Trend analysis performed after combination of female and male data for urinary bladder lesions from this same study (Arnold *et al.*, 2006).
[d] Short communication of tumour data only.
[e] On a C57BL/6 background.
[f] As stated by the authors.
[g] The lack of information on group size and the lack of descriptive statistics makes these data impossible to independently re-evaluate for statistical significance.
bw, body weight; F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

Table 3.3 Studies of cancer in experimental animals exposed to trimethylarsine oxide (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 2 yr Shen *et al.* (2003)	0, 50, 200 ppm trimethylarsine oxide in drinking-water, *ad libitum* 42–45; 42 controls	Liver (adenomas): 6/42 (9%), 10/42 (14%), 16/45 (24%)	$P < 0.05$ (high dose)	Age at start, 10 wk Purity, 99% Body weights, food intake, water intake, survival rate, and average survival unaltered with treatment Extensive necropsy performed Various other sites negative

bw, body weight; M, male; yr, year or years

3.3 Intravenous administration

3.3.1 Mouse

Multiple intravenous injections of sodium arsenate in male and female Swiss mice provided no evidence of elevated tumour formation (Waalkes *et al.*, 2000; see Table 3.8).

3.4 Transplacental and perinatal exposures

3.4.1 Mouse

Pregnant mice were treated subcutaneously with arsenic trioxide on a single specific day during gestation (Days 14, 15, 16 or 17), and the offspring were then treated subcutaneously on *postpartum* Days 1, 2 and 3 with arsenic trioxide. The offspring initially treated on Day 15 of gestation developed an excess of lung adenoma compared to controls, and the other groups did not (Rudnai & Borzsanyi, 1980, 1981; see Table 3.9).

Pregnant C3H mice were exposed to various doses of sodium arsenite in the drinking-water from Days 8–18 of gestation. They were allowed to give birth and their offspring were put into gender-based groups at weaning. Over the next 90 weeks, arsenic-treated female offspring developed dose-related benign and/or malignant ovarian tumours, and lung adenocarcinoma. During the next 74 weeks, a dose-related increase in the incidences of liver adenoma and/or carcinoma, and adrenal cortical adenoma was observed in the male offspring (Waalkes *et al.*, 2003).

A second study looked at the carcinogenic effects in C3H mice of various doses of sodium arsenite (two levels) in the maternal drinking-water from Days 8 to 18 of gestation, with or without subsequent 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) applied to the skin of the offspring after weaning from 4–25 weeks of age. Over the next 2 years, with arsenic alone, the female offspring developed an increased incidence of ovarian tumours. The male offspring developed arsenic dose-related increases in the incidences of liver adenoma and/or carcinoma and adrenal cortical adenoma (Waalkes *et al.*, 2004).

Pregnant CD1 mice received sodium arsenite (one level) in the drinking-water from gestation Days 8 to 18, were allowed to give birth, and the female (Waalkes *et al.*, 2006a) or male (Waalkes *et al.*, 2006b) offspring were treated with diethylstilbestrol or tamoxifen subcutaneously on *postpartum* Days 1, 2, 3, 4 and 5. In female offspring over the next 90 weeks, arsenic exposure alone

Table 3.4 Studies of cancer in experimental animals exposed to monomethylarsonic acid, MMA^V (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 104 wk Arnold *et al.* (2003)	0, 10, 50, 200, 400 ppm MMA^V in feed, *ad libitum* 52/group/sex	No treatment-related changes		Age at start, 6 wk Purity, 99% Bw reduced at 400 ppm throughout study Food and water consumption similar or increased at the two higher doses Survival unremarkable Complete necropsy performed
Rat, F344 (M, F) 104 wk Arnold *et al.* (2003)	0, 50, 400, 1 300[a] ppm MMA^V in feed, *ad libitum* 60/group/sex	No treatment-related changes		Age at start, 6 wk Purity, 99% Bw reduced at two highest doses in second half of study Food consumption generally similar Water consumption similar or increased at the two higher doses Survival reduced at high dose Complete necropsy performed

[a] Due to a high mortality in male and female rats fed this level, it was reduced to 1000 ppm during Week 53, and further reduced to 800 ppm during Week 60.
bw, body weight; F, female; M, male; wk, week or weeks

Table 3.5 Studies of cancer in experimental animals exposed to sodium arsenite (oral exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 167 wk (lifespan) Soffritti et al. (2006)	0, 50, 100, 200 mg/L $NaAsO_2$ in drinking-water, *ad libitum* from onset to 104 wk 50/group	Kidney (tumours): F– 1/50 (2%), 1/50 (2%), 5/50 (10%), 5/50 (10%)[c] M– 0/50, 2/50 (4%), 2/50 (4%), 0/50	NS for both sexes	Age at start, 8 wk Purity 98% Complete necropsy performed Reduced water and food intake especially at two highest doses Dose-related reduced bw

[a] As stated by the authors.
[b] The lack of information on group size and lack of descriptive statistics makes the data from this work impossible to re-evaluate for statistical significance.
[c] Includes three carcinomas at the high dose and one at the second highest dose in females and a carcinoma in females at the second highest dose.
Bw, body weight; F, female; M, male; NS, not significant; wk, week or weeks

increased the incidence of tumours of the ovary, uterus, and adrenal cortex. In the male offspring, prenatal arsenic exposure alone increased liver adenoma and/or carcinoma, lung adenocarcinoma, and adrenal cortical adenoma (see Table 3.10).

3.5 Studies in which arsenic modifies the effects of other agents

3.5.1 Mouse

Mice exposed to DMA^V in drinking-water after subcutaneous injection of 4-nitroquinoline 1-oxide showed an increase in lung tumour multiplicity compared to mice exposed to the organic carcinogen alone (Yamanaka et al., 1996). In K6/ODC mice first treated topically with 7,12-dimethylbenz[α]anthracene (DMBA) then with DMA^V in a cream applied to the same skin area for 18 weeks, the organo-arsenical doubled the skin tumour multiplicity compared to treatment with DMBA alone (Morikawa et al., 2000; see Table 3.11). [The Working Group noted that this study had too few DMA^V controls for an appropriate interpretation.]

In the studies of Germolec et al. (1997, 1998), oral sodium arsenite was given to Tg.AC mice with TPA by skin painting, and an approximately 4-fold increase in skin tumour response was reported.

Combined treatment with oral sodium arsenite in drinking-water and multiple exposures to excess topical UV irradiation in Crl:SKl-*hr*BR hairless mice showed that arsenic treatment alone was consistently without carcinogenic effect, but markedly enhanced UV-induced skin tumours including squamous cell carcinoma (Rossman et al., 2001; Burns et al., 2004; Uddin et al., 2005). In another skin study, mice exposed to topical 9,10-dimethyl-1,2-benzanthracene for 2 weeks concurrently with oral sodium arsenate in drinking-water for 25 weeks showed that arsenic treatment alone was without carcinogenic effect, but enhanced skin tumour multiplicity and tumour size when combined with the organic carcinogen compared to the organic carcinogen alone (Motiwale et al., 2005; see Table 3.12).

When pregnant Tg.AC mice were treated with oral sodium arsenite in drinking-water from Days 8–18 of gestation, and their offspring were topically exposed to TPA from 4–40 weeks

Table 3.6 Studies of cancer in experimental animals exposed to calcium arsenate (intratracheal instillation)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (M) ~145 wk (lifespan) Pershagen & Björklund (1985)	0, ~3 mg As/kg bw in 0.15 mL saline once/wk for 15 wk 41; 29 controls	Lung (adenomas): 0/26, 4/35 (11%)	$P < 0.05$	Age at start, 8 wk Purity, ultrapure Mortality during dosing ~15%; mortality increased in arsenate group during second yr Dose approximate
Hamster, Syrian golden (M) Up to 115 wk in treated animals, and 121 wk in controls (lifespan) Yamamoto *et al.* (1987)	0, 0.25 mg As in 0.1 mL saline once/wk for 15 wk 30; 22 controls	Lung (adenomas): 0/22, 6/25 (24%) Lung (carcinomas): 1/22 (4%), 1/25 (4%) Lung (adenomas and carcinomas combined): 1/22 (4%), 7/25 (3%)	[$P < 0.01$[a]] NS *P*-value not reported but stated as significant [$P < 0.01$[a]]	Age at start, 8 wk Purity, chemical grade Instillations caused 10% mortality and reduced survival ~10% post-instillation Bw not recorded during experiment

[a] Calculated by the Working Group. One-sided Fisher exact test control versus treated.
bw, body weight; M, male; NS, not significant; wk, week or weeks

Table 3.7 Studies of cancer in experimental animals exposed to arsenic trioxide (intratracheal instillation)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (M) Up to ~140 wk (lifespan) Pershagen *et al.* (1984)[a]	0 or ~3 mg As/kg bw in 0.15 mL saline once/wk for 15 wk 67; 68 controls	Larynx, trachea, bronchus, or lung (carcinomas): 0/53, 3/47 (6%) Larynx, trachea, bronchus, or lung (adenomas, adenomatoid lesions, and papillomas combined): 7/53 (13%), 24/47 (51%)	NS [$P < 0.01$]	Age at start, 7–9 wk Purity, 99.5% Doses approximate Instillation mixture for arsenic contained carbon dust and 2 mM sulfuric acid (not in controls) Significant mortality during dosing (29%) “Adenomatoid lesion” not defined, presumably focal hyperplasia

[a] Arsenic trioxide was also given with benzo[*a*]pyrene and the combination appeared to increase combined adenoma, adenocarcinoma and adenosquamous carcinoma in the bronchi and lungs compared to benzo[*a*]pyrene alone but the data are listed (total tumours/group and not incidence) such that this cannot be independently confirmed.
bw, body weight; M, male; NS, not significant; wk, week or weeks

Table 3.8 Studies of cancer in experimental animals exposed to sodium arsenate (intravenous exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss CR:NIH(S) (M, F) 96 wk Waalkes et al. (2000)	0, 0.5 mg As/kg bw in 10 mL/kg in saline once/wk for 20 wk staring at onset; controls received saline[a] 25/group/sex	**M** Lymphomas: 1/25 (4%), 1/25 (4%) Testicular interstitial cell hyperplasias: 8/25 (32%), 16/25 (64%) Skin hyperkeratosis: 1/25 (4%), 5/25 (20%) **F** Lymphomas: 5/25 (20%), 3/25 (12%) Uterine cystic hyperplasias: 5/25 (20%), 14/25 (56%)[b]	 NS $P < 0.05$ NS NS $P < 0.05$	Age at start, 8 wk Purity, NR Survival and bw not remarkable No leukaemias were observed

[a] Based on the treatment regimen of Osswald & Goerttler (1971).

[b] A uterine adenocarcinoma was also observed with arsenate treatment that is noteworthy because of its spontaneous rarity in historical controls of this strain.

bw, body weight; F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

Table 3.9 Studies of cancer in experimental animals exposed to arsenic trioxide (perinatal exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CFLP (NR) 1 yr Rudnai & Borzsanyi (1980), Rudnai & Borzsanyi (1981)[a]	Single dose of 1.2 mg/kg arsenic trioxide bw s.c. at gestation Day 14, 15, 16, or 17 Test offspring: 5 µg arsenic trioxide/mouse s.c. postpartum Day 1, 2 and 3 Controls untreated Offspring group sizes at start (NR)	Lung (adenomas and adenocarcinomas):[b] Control–3/17 (17%) Day 14–14/36 (39%) Day 15–12/19 (63%) Day 16–3/20 (15%) Day 17–6/20 (30%)	$P < 0.01$ (Day 15)[b]	Purity stated as "purum" Pregnancy verified by smear and when positive designated Day 0 Dam number used to derive offspring groups NR Lung and gross lesions histologically examined Survival and bw NR Gender NR and probably mixed Numbers of specific lung tumours NR

[a] In Hungarian. Tumour incidence data are numerically the same for this and the Rudnai & Borzsanyi (1980) manuscript, but vary in that the treatment day of pregnancy which lead to a significant increase in lung adenoma in the first paper (Day 15) shifted to one day later in the second paper (Day 16). Communication with the primary author revealed that this discrepancy in the re-reporting (Rudnai & Borzsanyi, 1981) is due to a difference in calling the first day on which pregnancy was indicated Day 1 of gestation rather than Day 0 as in the original report (Rudnai & Borzsanyi, 1980). Thus, the treatment regimen and data from the primary paper are herein reported.

[b] The gestational treatment day is given in parentheses before incidence or after indication of significance.

bw, body weight; NR, not reported; s.c., subcutaneously; yr, year or years

Table 3.10 Studies of cancer in experimental animals exposed to sodium arsenite (transplacental exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C3H/HeNCr (M, F) 90 wk (*postpartum*) for F 74 wk (*postpartum*) for M Waalkes *et al.* (2003)	Maternal exposure: 0, 42.5, 85 ppm As in drinking-water, *ad libitum* from gestation Day 8–18 Offspring; 25/group/sex	**Females** Ovary (tumours): Benign–2/25 (8%), 4/23 (17%), 8/24 (33%) Malignant–0/25, 2/23 (9%), 1/24 (4%) Benign or malignant combined–2/25 (8%), 6/23 (26%), 9/24 (37%) Lung (carcinomas): 0/25, 1/23 (4%), 5/24 (20%) **Males** Liver (adenomas): 9/24 (37%), 9/21 (43%), 20/23 (87%) Liver (hepatocellular carcinomas): 2/24 (8%), 8/21 (38%), 14/23 (61%) Liver (adenomas or hepatocellular carcinomas): 10/24 (42%), 11/21 (52%), 20/23 (87%) Liver tumours/mouse: 0.87, 1.81, 4.91 Adrenal cortex (adenomas): 9/24 (37%), 14/21 (67%), 21/23 (91%) Adrenal adenomas/mouse: 0.71, 1.10, 1.57	$P < 0.05$ (high dose plus trend) NS $P < 0.05$ (high dose) $P < 0.05$ (trend) $P < 0.05$ (high dose) $P < 0.05$ (trend) $P < 0.01$ (high dose) $P < 0.05$ (high dose) $P < 0.01$ (trend) $P < 0.05$ (high dose) $P < 0.01$ (trend) $P < 0.05$ (high dose) $P < 0.01$ (trend) $P < 0.05$ (high dose) $P < 0.01$ (trend) $P < 0.05$ (high dose) $P < 0.05$ (trend)	Purity,[a] NR 10 Pregnant mice used to derive each group of offspring Offspring weaned at 4 wk Maternal water consumption and bw unaltered Offspring bw unaltered Survival in offspring unaltered in females Survival reduced at high dose in due to liver carcinoma in males

Table 3.10 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C3H/HeNCr (M, F) 104 wk (*postpartum*) Waalkes *et al.* (2004)	Maternal exposure: 0, 42.5, 85 ppm As in drinking-water, *ad libitum* from gestation Day 8–18 Offspring exposure: topical 2 µg[b] TPA/0.1 mL acetone, twice/wk from 4–25 wk of age applied to shaved back, controls received acetone Offspring groups: 25/group/sex	**Females** Liver (adenomas or hepatocellular carcinomas):		Purity,[a] NR 10 Pregnant mice used to derive each group of offspring Litters culled at 4 d *postpartum* to no more than 8 pups Maternal water consumption and bw unaltered Small bw reductions (~10%) occurred late (> 95 wk) in the high-dose (85 ppm) female offspring TPA did not alter bw Survival unaltered Inclusion of TPA did not have an impact on skin cancers
		Without TPA–3/24 (12%), 6/23 (26%), 4/21 (19%)	NS	
		With TPA–3/24 (12%), 6/22 (27%), 8/21 (38%)	$P < 0.05$ (high dose and trend)	
		Liver tumour multiplicity (tumours/mouse):		
		Without TPA–0.13, 0.41, 0.29	NS	
		With TPA–0.13, 0.32, 0.71	$P < 0.05$ (high dose and trend)	
		Ovary (tumours):[c]		
		Without TPA–0/24, 5/23 (22%), 4/21 (19%)	$P < 0.05$ (both doses)	
		With TPA–0/24, 5/22 (23%), 4/21 (19%)	$P < 0.05$ (both doses)	
		Lung (adenomas):		
		Without TPA–1/24 (4%), 2/23 (9%), 2/21 (9%)	NS	
		With TPA–1/24 (4%), 2/22 (9%), 6/21 (29%)	$P < 0.05$ (high dose and trend)	
		Males Liver (tumours):		Arsenic group not given TPA due to liver carcinoma (males)
		Adenomas without TPA–10/24 (42%), 12/23 (52%), 19/21 (90%)	$P < 0.05$ (high dose) $P < 0.01$ (trend)	
		Adenomas with TPA–8/23 (35%), 12/23 (52%), 16/21 (76%)	$P < 0.05$ (high dose) $P < 0.01$ (trend)	
		Hepatocellular carcinomas without TPA–3/24 (12%), 8/23 (35%), 10/21 (48%)	$P < 0.05$ (high dose) $P < 0.01$ (trend)	
		Hepatocellular carcinomas with TPA–2/23 (9%), 6/23 (26%), 7/21 (33%)	$P < 0.05$ (high dose and trend)	
		Adenomas or hepatocellular carcinomas without TPA–12/24 (50%), 14/23 (52%), 19/21 (90%)	$P < 0.05$ (high dose) $P < 0.01$ (trend)	

Table 3.10 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Waalkes et al. (2004) (contd.)		Adenomas or hepatocellular carcinomas with TPA–9/23 (39%), 15/23 (65%), 18/21 (90%) Multiplicity without TPA: 0.75, 1.87, 2.14 Multiplicity with TPA: 0.61, 1.44, 2.14 Adrenal cortex (adenomas): Without TPA–9/24 (37%), 15/23 (65%), 15/21 (71%) With TPA–7/23 (30%), 15/23 (65%), 12/21 (57%) Lung (adenomas): Without TPA–4/24 (17%), 6/23 (26%), 5/21 (24%) With TPA–2/23 (9%), 10/23 (43%), 5/21 (24%)	$P < 0.05$ (high dose) $P < 0.01$ (trend) $P < 0.05$ (both doses) $P < 0.01$ (trend) $P < 0.05$ (both doses) $P < 0.01$ (trend) $P < 0.05$ (high dose and trend) $P < 0.05$ (low dose) NS $P < 0.05$ (low dose)	
Mouse, CD1 (M, F) 90 wk (*postpartum*) Waalkes et al. (2006a, b)[k]	Maternal exposure: 0, 85 ppm As in drinking-water, *ad libitum* from gestation Day 8–18 Offspring exposure: *Postpartum* Day 1, 2, 3, 4, and 5 2 µg DES[d]/pup/d s.c., or 10 µg TAM[e]/pup/d s.c., or vehicle (corn oil; control) (control, As, DES, TAM, As + DES, As + TAM) 35/group/sex	**Females** Ovary (tumours):[h] 0/33, 7/34 (21%), 2/33 (6%), 1/35 (3%), 9/33 (26%), 5/35 (14%) Uterus (adenomas): 0/33, 3/34 (9%), 0/33, 0/35, 0/33, 0/35 Uterus (carcinomas): 0/33, 2/34 (6%), 0/33, 2/35 (6%), 7/33 (21%), 2/35 (6%) Uterus (adenomas or carcinomas): 0/33, 5/34 (15%), 0/33, 2/35 (6%), 7/33 (21%), 2/35 (6%) Vagina (carcinomas): 0/33, 0/34, 1/33, 0/35, 5/33[g] (15%), 0/35 Adrenal cortex (adenomas): 1/33 (3%), 9/34 (26%), 3/33 (9%), 2/35 (6%), 8/33 (24%), 7/35 (20%) Urinary bladder lesions:	$P < 0.05$ (As, As + DES, As + TAM) NS $P < 0.05$ (As + DES) $P < 0.05$ (As, As + DES) $P < 0.05$ (As + DES) $P < 0.05$ (As, As + DES, As + TAM)	Purity 97.0% $NaAsO_2$ 12 Pregnant mice used to derive each group of offspring Litters culled after birth to no more than 8 pups Maternal water consumption unaltered Maternal and offspring bw unaltered

Table 3.10 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Waalkes *et al.* (2006a, b) (contd.)		Hyperplasias– 1/33 (3%), 5/34 (15%), 1/33 (3%), 0/35, 10/33 (30%), 9/35 (26%)	$P < 0.05$ (As + DES, As + TAM)	
		Papillomas– 0/33, 0/34, 0/33, 0/35, 0/33, 1/35 (3%)	NS	
		Carcinomas[j]– 0/33, 0/34, 0/33, 0/35, 3/33 (9%), 0/35	NS	
		Total proliferative lesions[j]– 1/33 (3%), 5/34 (15%), 1/33 (3%), 0/35, 13/33[g] (38%), 10/35[g] (29%)	$P < 0.05$ (As + DES, As + TAM)	
		Liver (tumours any type): 0/33, 4/34 (12%), 1/33 (3%), 0/35, 5/33 (15%), 4/35 (11%)	$P < 0.05$ (As + DES)	
		Males Liver (tumours): Adenomas– 2/35 (6%), 8/35 (23%), 1/33 (3%), 0/30, 12/29 (41%), 9/30 (30%)	$P < 0.05$ (As, As + DES, As + TAM)	Purity sodium arsenite 97.0%; DES 99%, TAM 99% Bw transiently reduced (~15%) by DES or TAM early but recovery to control levels by 5–20 wk *postpartum* Survival unaltered by prenatal arsenic alone. Survival reduced in all other treatment groups (DES, TAM, As + DES, As + TAM) from ~20 wk on compared to control[l] (males)
		Hepatocellular carcinomas– 0/35, 5/35 (14%), 0/33, 0/30, 4/29 (14%), 5/30 (17%)	$P < 0.05$ (As, As + DES, As + TAM)	
		Adenomas or carcinomas– 2/35 (6%), 11/35 (31%), 1/33 (3%), 0/30, 14/29 (48%), 14/30 (47%)	$P < 0.05$ (As, As + DES,)	
		Lung (adenocarcinomas): 2/35 (6%), 9/35 (26%), 2/33 (6%), 0/30, 4/29 (14%), 6/30 (20%)	$P < 0.05$ (As)	
		Adrenal cortex (adenomas): 0/35, 13/35 (37%), 0/33, 0/30, 9/29 (31%), 11/30 (37%)	$P < 0.05$ (As, As + DES, As + TAM)	
		Urinary bladder lesions: Hyperplasias– 0/35, 3/35 (9%), 4/33 (12%), 3/30 (10%), 13/29[g] (45%), 9/30[g] (30%)	$P < 0.05$ (As + DES, As + TAM)	
		Papillomas– 0/35, 0/35, 0/33, 0/30, 0/29, 3/30 (10%)	NS	

Table 3.10 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Waalkes *et al.* (2006a, b) (contd.)		Carcinomas[i]– 0/35, 0/35, 0/33, 0/30, 1/29 (3%), 1/30 (3%)	NS	
		Papillomas or carcinomas– 0/35, 0/35, 0/33, 0/30, 1/29 (3%), 4/30[g] (13%)	$P < 0.05$ (As + TAM)	
		Total proliferative lesions[j]– 0/35, 3/35 (9%), 4/33 (12%), 3/30 (10%), 13/29[g] (45%), 14/30[g] (40%)	$P < 0.05$ (As + DES, As + TAM)	

[a] Purity given in Waalkes *et al.* (2006a) using same chemical source is 97.0%.
[b] 12-*O*-tetradecanoyl phorbol-13-acetate.
[c] Exclusively epithelial and primarily adenoma.
[d] Diethylstilbestrol
[e] Tamoxifen
[f] Included benign and malignant epithelial and mesenchymal tumours within components of the urogenital system (ovary, oviduct, uterus, cervix, vagina, kidney, and urinary bladder).
[g] Incidence for arsenic plus DES or arsenic plus TAM was significantly ($P < 0.05$) higher than arsenic alone.
[h] Primarily adenoma.
[i] Exclusively transitional cell carcinoma.
[j] Defined by the authors as the incidence of mice bearing at least one uroepithelial preneoplasia (hyperplasia), papilloma, or carcinoma.
[k] Run concurrently with and derived from the same mothers as the females in Waalkes *et al.* (2006a) study but reported separately.
[l] Reduced survival in these groups appeared dependent on moderate to extensive kidney damage due to DES and TAM in male mice and appeared unrelated to arsenic exposure.
[m] Two renal tumours also occurred in this group including, an adenoma and a renal cell carcinoma, against none in control, which are noteworthy because of their rare spontaneous occurrence in mice.
d, day or days; DES, diethylstilbestrol; F, female; M, male; NR, not reported; NS, not significant, s.c., subcutaneously; TAM, tamoxifen; wk, week or weeks

Table 3.11 Studies where arsenicals given after other agents enhance carcinogenesis while having no effect alone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ddy (M) 25 wk Yamanaka *et al.* (1996)	Initiation 10 mg 4NQO[e]/kg bw s.c. then 200 or 400 ppm DMA^V in drinking-water for 25 wk Groups: 4NQO alone, 4NQO + 200 ppm DMA, 4NQO + 400 ppm DMA 9–13/group	Macroscopic lung tumours/mouse: 0.22, 3.92, 4.38	$P < 0.05$ (high dose)	Age at start, 6 wk DMA^V purity, NR Bw and survival unremarkable DMA^V alone group not included Lung only Microscopic analysis of lung tumours not reported (largely confirmed as tumours) Small group sizes
Mouse, *K6/ODC* (C57BL/6J background) 20 wk Morikawa *et al.* (2000)	Single 50 µg dose of DMBA[f]/mouse topical dorsal skin at Week 1; then 3.6 mg DMA^V/mouse in "neutral cream" to dorsal skin twice/wk, Week 2–19 Groups: DMBA, DMBA + DMA^V 7; 8 controls (DMBA)	Macroscopic skin tumours/mouse: 9.7, 19.4	$P < 0.05$	Age at start, 10–14 wk DMA^V purity, NR Bw and survival unremarkable DMA^V-alone group had only 2 mice; skin tumours not reported Small group sizes Skin only No quantitative microscopic analysis of skin tumours
Rat, Wistar (M) 175 d Shirachi *et al.* (1983)	Sodium arsenite Partial heptectomy, 18–24 h later 30 mg DEN[a]/kg i.p.; 7 d later 160 ppm As in drinking-water Number at start, NR	Renal tumours: 0/10, 1/7 (14%), 0/9, 7/10 (70%)	$P < 0.05$	Age at start, NR Purity, NR Arsenic lowered bw and water intake Limited reporting and never reported in full

Table 3.11 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 30 wk Yamamoto *et al.* (1995)	Initial pretreatment with 5 known carcinogens (termed DMBDD[b]) then 0, 50, 100, 200, 400 ppm DMA^V in the drinking-water during Week 6–30 Groups: DMBDD alone, DMBDD + 50 ppm DMA^V, DMBDD + 100 ppm DMA^V, DMBDD + 200 ppm DMA^V, DMBDD + 400 ppm DMA^V 20/group	Urinary bladder:[c] Papillomas– 1/20 (5%), 12/20 (60%), 12/19 (63%), 11/20 (55%), 7/20 (35%) Transitional cell carcinomas– 1/20 (5%), 10/20 (50%), 11/19 (60%), 12/20 (60%), 13/20 (65%) Papillomas or carcinomas– 2/20 (10%), 17/20 (85%), 16/19 (84%), 17/20 (85%), 16/20 (80%) Kidney: Adenomas– 1/20 (5%), 3/20 (15%), 1/19 (5%), 7/20 (35%), 3/20 (15%) Adenocarcinomas– 0/20, 0/20, 2/19 (10%), 1/20 (5%), 7/20 (35%) Total– 5/20 (25%), 3/20 (15%), 6/19 (30%), 13/20 (65%), 13/20 (65%) Liver: Hepatocellular carcinomas– 0/20, 2/20 (10%), 0/19, 8/20 (40%), 8/20 (40%) Total– 0/20, 2/20 (10%), 2/19 (10%), 17/20 (85%), 13/20 (65%) Total thyroid gland tumours: 3/20 (15%), 2/20 (10%), 8/19 (40%), 6/20 (30%), 9/20 (45%)	 $P < 0.01$ (three lowest) $P < 0.05$ (highest) $P < 0.01$ (all DMA^V treatment groups) $P < 0.01$ (all DMA^V treatment groups) $P < 0.01$ (second highest) $P < 0.01$ (high dose and trend) $P < 0.05$ (trend) $P < 0.05$ (highest two and trend) $P < 0.05$ (highest two) $P < 0.01$ (trend) $P < 0.05$ (highest) $P < 0.01$ (trend)	Age at start, 7 wk DMA^V purity, 99%; DMA^V initially lowered but then increased bw; changes moderate and at high dose DMA^V increased water intake at high dose Survival unremarkable Separate 100 and 400 ppm (12 each) DMA^V alone groups were included but had no tumours or preneoplastic lesions

Table 3.11 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 36 wk Wanibuchi *et al.* (1996)	Pretreatment with BBN[d] 0.05% in drinking-water for 4 wk then 0, 2, 10, 25, 50, or 100 ppm DMA^V in drinking-water for 32 wk Groups: BBN alone, BBN + 2 ppm DMA^V, BBN + 10 ppm DMA^V, BBN + 50 ppm DMA^V, BBN + 100 ppm DMA^V 20/group	Urinary bladder: Papillary/nodular hyperplasias– 14/20 (70%), 13/20 (65%), 14/20 (70%), 18/19 (95%), 20/20 (100%), 20/20 (100%) Papillomas– 3/20 (15%), 2/20 (10%), 7/20 (35%), 11/19 (58%), 13/20 (65%), 17/20 (85%) Carcinomas– 1/20 (5%), 2/20 (10%), 3/20 (15%), 7/19 (37%), 10/20 (50%), 12/20 (60%)	$P < 0.05$ (highest two doses) $P < 0.01$ (highest three doses) $P < 0.05$ (third highest dose) $P < 0.01$ (highest two doses)	Age at start, ~6 wk DMA^V purity, 99% Separate 0 and 100 ppm control and DMA^V alone groups were included (12 each) but showed no urinary bladder tumours or preneoplastic lesions Bw, water intake and survival unremarkable Urinary bladder only

[a] Diethylnitrosamine

[b] The organic carcinogen treatment consisted of a single dose of diethylnitrosamine (100 mg/kg, i.p.) at the start of the experiment) and*N*-methyl-*N*-nitrosourea (20 mg/kg, s.c.) on experimental Days 5, 8, 11 and 14. Thereafter, rats received 1,2-dimethylhydrazine (40 mg/kg, s.c.) on Days 18, 22, 26, and 30). During the same period (experimental Days 0–30) the rats received *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (0.05% in the drinking-water Weeks 1 and 2) and *N*-bis(2-hydroxypropyl)nitrosamine (0.1% in the drinking-water, Weeks 3 and 4). Altogether this was defined as DMBDD treatment. Rats received no treatment for 2 wk after DMBDD exposure and before DMA exposure.

[c] For brevity, only significant proliferative lesions are noted for each tissue

[d] *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine

[e] 4-Nitroquinoline

[f] 7,12-dimethylbenz[α]anthracene

[g] Estimated from graphical presentation.

d, day or days; DMA, dimethylarsinic acid; F, female; i.p., intraperitoneal; M, male; NR, not reported; s.c., subcutaneously; wk, week or weeks

Table 3.12 Studies where arsenicals given concurrently with other agents enhance carcinogenesis while having no effect alone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Tg.AC homozygous (F) 14 wk Germolec *et al.* (1997)	0 or 0.02% As in drinking-water, *ad libitum* throughout experiment 0 or 2.5 µg TPA[a]/mouse in acetone topical to shaved dorsal skin twice/wk, Week 5 and 6 Groups: control, As alone, TPA, As + TPA 20/group	Macroscopic skin papillomas/mouse: none in control or arsenic alone, intermediate in TPA alone (~0.5/mouse),[b] "4-fold higher" (~2.1/mouse)[b] in arsenic + TPA	NR	Age at start, NR Purity, NR Survival unremarkable Specific quantitative microscopic analysis of skin tumours not included but confirmed as papillomas at termination Skin lesions only Incomplete reporting makes independent statistical analysis impossible
Mouse, Tg.AC homozygous (F) 24 wk Germolec *et al.* (1998)	0 or 0.02% As in drinking-water, *ad libitum* throughout experiment 0, 1.25, 2.5 µg TPA/mouse in acetone topical to shaved dorsal skin twice/wk, Week 5 and 6 Groups: control, As alone, 1.25 TPA, 2.5 TPA, As + 1.25 TPA, As + 2.5 TPA 20/group	Macroscopic skin papillomas/mouse: 0 in control, As alone, and 1.25 TPA alone; As + 1.25 TPA maximal ~5/mouse,[b] 2.5 TPA ~3/mouse,[b] in arsenic + 2.5 TPA ~7/mouse[b]	NR	Age at start, 8 wk Purity, NR Survival impacted by high-dose TPA co-treatment but specifics not given Quantitative microscopic analysis of skin tumours not included but confirmed as papillomas at termination Skin lesions only Incomplete reporting makes independent statistical analysis impossible

JNJ 000451378

Table 3.12 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Crl: SKl-*hr*BR (hairless) (F) 29 wk Rossman *et al.* (2001)	0, 10 mg/L sodium arsenite in drinking-water throughout experiment plus topical 1.7 kJ/m² solar irradiation (85% UVB, < 1% UVC, 4% UVA, remainder visible; termed UVR[c]) 3x/wk starting 3 wk after As until termination Groups: control, As alone, UVR alone, As + UVR 5–15; 5 controls	Skin (tumours): Macroscopic and microscopic analysis–0/5, 0/5 (control and As alone) Macroscopic analysis– Time to first occurrence: As + UVR earlier than UVR Microscopic analysis– Total tumours all mice: 53 (UVR), 127 (As + UVR) Highly invasive squamous cell carcinoma: 14/53 (26%; UVR), 64/127 (50%; As + UVR) Tumour volume: UVR smaller than As + UVR	 $P < 0.01$ $P < 0.01$ $P < 0.01$	Age at start, 3wk Purity, NR Survival and bw unremarkable Small control groups
Mouse, SKl (hairless), (NR) 29 wk Burns *et al.* (2004)	Experiment 1: 0, 1.25, 2.50, 5.00, 10.0 mg/L sodium arsenite in drinking-water from onset plus topical 0 or 1.0 kJ/m² solar irradiation (UVR[c]) 3x/wk, starting 3 wk after As to termination Experiment 2: 10.0 mg/L sodium arsenite in drinking-water from onset plus topical 1.7 kJ/m² UVR[c] 3x/wk starting 3 wk after As to termination	Experiment 1: Skin tumours/mouse[d]: 2.4 (UVR), 5.4 (1.25 As + UVR), 7.21 (2.5 As + UVR), 11.1 (5.0 As + UVR), 6.8 (10.0 As + UVR) Experiment 2: Skin tumours/mouse:[d] 3.5 (UVR), 9.6 (As + UVR) Skin tumour incidence: 0/10, 0/10 (control and As alone both experiments)	[$P < 0.01$ all groups vs UVR alone[e]] [$P < 0.01$[f]]	Age, 3 wk Survival and bw unremarkable Specific quantitative microscopic analysis of skin tumours not reported but confirmed as primarily squamous cell carcinomas at termination Experiment 1 shows clear arsenic dose–response in enhancement through 5.0 mg/L by various criteria

Table 3.12 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Crl: SKl-*hr*BR (hairless) (F) Duration, NR Uddin *et al.* (2005)	0, 5 mg/L sodium arsenite in drinking-water from onset; diet unsupplemented or with added vitamin E (62.5 IU/kg diet; basal 49.0 IU/kg) or *p*-XSC[g] (10 mg/kg diet) from onset. Topical 1.0 kJ/m² UVR[c] 3x/wk starting 3 wk after As to termination. Groups: UVR alone, UVR + As, UVR + As + Vitamin E, UVR + As + *p*-XSC[h] 10; 30 controls (UVR)	Macroscopic skin tumours/mouse: 3.60 (UVR alone), 7.00 (UVR + As), 3.27 (UVR + As + Vitamin E), 3.40 (UVR + As + *p*-XSC)	$P < 0.01$ (UVR vs UVR + As) $P < 0.01$ (UVR + As vs UVR + As + either dietary supplement)	Age at start, 3 wk Sodium arsenite, purity (NR), *p*-XSC Purity > 99% Survival and bw unremarkable Small control groups Vitamin E and *p*-XSC added as antioxidants Specific quantitative microscopic analysis of skin tumours not reported but random sampling (10 tumours/group) confirmed primarily squamous cell carcinomas at termination No untreated control or arsenic alone groups included
Mouse, Swiss-bald hairless (M) 25 wk Motiwale *et al.* (2005)	Treatment with 2 mg BA[i] /mL 25 µL topical once/wk for 2 wk Sodium arsenate 0 or 25 mg/L drinking-water for 25 wk Groups: Control, BA, As, BA + As 10/group	Macroscopic skin tumours/mouse: 0, 2.0, 0, 3.2[b] % large papillomas (≥ 3 mm) of total papillomas: 0, 16, 0, 65[d]	$P < 0.05$ (As + BA vs BA) $P < 0.05$ (As + BA vs BA)	Age at start, 8 wk Purity, NR Survival unremarkable Small group sizes Quantitative microscopic skin tumour incidence or multiplicity not reported though histologically confirmed

[a] 12-*O*-tetradecanoyl-13-acetate.
[b] Estimated from graphical presentation. No descriptive statistics included.
[c] UVR as defined in Rossman *et al.* (2001) above.
[d] Data included descriptive statistics.
[e] Using Dunnett's multiple comparison test and not including arsenic alone and untreated control groups
[f] Using Student's *t*-test.
[g] 1,4-Phenyl*bis*(methylene)selenocyanate a synthetic organoselenium compound.
[h] Some control groups are not discussed for the sake of brevity (UVR + Vitamin E and UVR +*p*-XSC).
[i] 9,10-dimethyl-1,2-benzanthracene.
F, female; M, male; NR, not reported; wk, week or weeks

of age, although arsenic treatment alone had no effect, it markedly increased the multiplicity of squamous cell carcinoma when combined with TPA compared to TPA alone (Waalkes *et al.*, 2008; see Table 3.13).

Prenatal sodium arsenite exposure via maternal drinking-water when combined with postnatal topical TPA exposure increased the liver tumour incidence and multiplicity in an arsenic-dose-related fashion (female offspring), and lung tumours (male offspring) compared to controls; effects not seen with TPA or arsenic alone (Waalkes *et al.*, 2004). Prenatal arsenic exposure followed by postnatal diethylstilbestrol increased uterine carcinoma, vaginal carcinoma, urinary bladder total proliferative lesions, and liver tumours in female offspring compared to controls; effects not seen with diethylstilbestrol or arsenic alone. In female offspring, prenatal arsenic exposure followed by postnatal tamoxifen administration similarly increased urinary bladder total proliferative lesions (Waalkes *et al.*, 2006a).

In male offspring, prenatal arsenic exposure followed by postnatal diethylstilbestrol increased the liver tumour response and urinary bladder total proliferative lesions effects when compared to controls; effects not seen with diethylstilbestrol or arsenic alone. In male offspring, prenatal arsenic exposure followed by postnatal tamoxifen increased liver tumour response, urinary bladder total tumours, and urinary bladder total proliferative lesions (Waalkes *et al.*, 2006b).

3.5.2 Rat

Rats that underwent partial hepatectomy followed by diethylnitrosamine injection and one week later by oral administration of sodium arsenite in the drinking-water for approximately 24 weeks showed an increased incidence of renal tumours, but arsenic treament alone had no effect (Shirachi *et al.*, 1983).

In a comprehensive study, rats were given an initial pretreatment with a mixture of organic carcinogens (including diethylnitrosamine, *N*-methyl-*N*-nitrosourea, 1,2-dimethylhydrazine, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, and *N*-bis(2-hydroxypropyl)nitrosamine) by various routes, no treatment for 2 weeks and then DMA^V (at four levels) in the drinking-water for 24 weeks, rats developed an increased incidence of tumours of urinary bladder with the combined carcinogen treatment and arsenical (Yamamoto *et al.*, 1995).

In another study in rats, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking-water was used as an initiator for 4 weeks followed by four levels of DMA^V for 32 weeks, and the combined treatment increased urinary bladder hyperplasia, papilloma, and carcinoma, but the arsenical treatment alone had no effect (Wanibuchi *et al.*, 1996).

3.6 Gallium arsenide

A single study (NTP, 2000) was judged to provide evidence for the carcinogenicity of gallium arsenide in rodents. In this report, $B6C3F_1$ mice and F344 rats were exposed via inhalation to various levels of gallium arsenide particulate for up to ~2 years, and the tumour response was assessed in various tissues (see Table 3.14).

3.6.1 Mouse

No treatment-related tumours were observed, but in both males and females, dose-related increases in the incidence in lung epithelial alveolar hyperplasia were reported.

3.6.2 Rat

In female rats, dose-related responses were reported for the incidence of lung alveolar/bronchiolar tumours and atypical hyperplasia

Table 3.13 Studies where arsenic given before another agent enhances carcinogenesis while having no effect alone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Tg.AC (M, F) Homozygous 40 wk (*postpartum*) Waalkes *et al.* (2008)	Maternal exposure: 0, 42.5, 85 ppm arsenic in drinking-water, *ad libitum*, gestation Day 8–18 Offspring exposure:[a] TPA, 2 µg/0.1 mL acetone, topical twice/wk, applied to shaved dorsal skin, 4–40 wk of age (36 wk of TPA exposure) Offspring groups (M, F):[c] Without TPA: (0, 42.5, 85 ppm arsenic) With TPA: (0, 42.5, 85 ppm arsenic) 50/group	Skin (tumours): Papillomas/mouse[a]– 0.5 (control), 0.9 (42.5 As), 0.12 (85 As), 17 (TPA[b]), 17 (42.5 As + TPA), 11 (85 As + TPA) Squamous cell carcinomas/mouse:[a] 0.04 (control), 0.06 (42.5 As), 0.04 (85 As), 0.57 (TPA), 1.31 (42.5 As + TPA), 1.49 (85 As + TPA) Incidence of mice with 3 or more squamous cell carcinomas: 0/49 (control), 0/47 (42.5 As), 0/48 (85 As), 1/47 (2%; TPA), 9/48 (19%; 42.5 As + TPA), 14/49 (29%; 85 As + TPA)	$P < 0.05$ (all TPA groups vs control; TPA alone vs 85As + TPA) $P < 0.05$ (all TPA groups vs control; all As + TPA groups vs TPA alone $P < 0.01$ (trend with As in TPA-treated mice) $P < 0.05$ (all TPA + As groups vs control or TPA alone) $P < 0.01$ (trend with As in TPA-treated mice)	Age, 4 wk (offspring) Purity, NR Litters culled at 4 d *postpartum* to no more than 8 pups 10 pregnant mice used to randomly derive each group Maternal water consumption and body unaltered Offspring weaned at 4 wk Offspring bw unaltered by arsenic All skin tumours were histopathologically diagnosed for stage and number per animal Some mice were killed because of tumour burden during experiment but were not lost to observation Only skin tumours reported

[a] Manuscript included descriptive statistics.
[b] 12-*O*-tetradecanoyl-13-acetate.
[c] Because initial analysis of tumours showed no gender-based differences between similarly treated groups of males and females, they were pooled for final assessment and are reported as such. Initial groups were made up of 25 M and 25 F mice.
bw, body weight; F, female; M, male; NR, not reported; vs; versus; wk, week or weeks

Table 3.14 Studies of cancer in experimental animals exposed to gallium arsenide (inhalation exposure)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 105 wk for M 106 wk for F NTP (2000)	0, 0.1, 0.5, 1.0 mg/m³ 6 h/d, 5 d/wk 50/group/sex	**Females** Lung (epithelial alveolar hyperplasias):2/50 (4%), 5/50 (10%), 27/50 (54%), 43/50 (86%) Lung[a] (adenomas or carcinomas): 7/50 (14%), 4/50 (8%), 4/50 (8%), 6/50 (12%) **Males** Lung (epithelial alveolar hyperplasias):4/50 (8%), 9/50 (18%), 39/50 (78%), 45/50 (90%) Lung[a] (adenomas or carcinomas): 15/50 (30%), 14/50 (28%), 16/50 (32%), 13/50 (26%)	 $P \leq 0.01$ (high dose) $P \leq 0.01$ (mid-dose) NS $P \leq 0.01$ (high dose) $P \leq 0.01$ (mid-dose) NS	Age at start, 6 wk Purity > 98% MMAD, 0.9–1.0 µm GSD, 1.8–1.9 µm Chamber controls used No reduced bw with treatment Survival unaltered No increases in tumour incidence
Rat, F344 (F) 105 wk NTP (2000)	0, 0.01, 0.1, 1.0 mg/m³ 6 h/d, 5 d/wk 50/group/sex	**Females** Lung[a] (adenomas): 0/50, 0/50, 2/50 (4%), 7/50 (14%) Lung (carcinomas): 0/50, 0/50, 2/50 (4%), 3/50 (6%) Lung (adenomas or carcinomas): 0/50, 0/50, 4/50 (8%), 9/50 (18%) Adrenal medulla:[b] 4/50 (8%), 6/49 (12%), 6/50 (12%), 13/49 (27%) Mononuclear cell leukaemia: 22/50 (44%), 21/50 (42%), 18/50 (36%), 33/50 (66%) **Males** Lung (atypical hyperplasias): 0/50, 2/49 (4%), 5/50 (10%), 18/50 (36%) Lung[a] (adenomas): 1/50 (2%), 0/49, 3/50 (6%), 2/50 (4%) Lung (carcinomas): 2/50 (4%), 0/49, 2/50 (4%), 1/50 (2%) Lung (adenomas or carcinomas): 3/50 (6%), 0/49, 5/50 (10%), 3/50 (6%)	 $P \leq 0.01$ (high dose) $P \leq 0.01$ (trend) NS $P \leq 0.01$ (high dose) $P \leq 0.01$ (trend) $P \leq 0.01$ (high dose) $P \leq 0.01$ (trend) $P \leq 0.05$ (high dose) $P \leq 0.01$ (trend) $P \leq 0.01$ (high dose) $P \leq 0.05$ (mid-dose) NS NS NS	Age at start, 6 wk Purity > 98% MMAD, 0.9–1.0 µm GSD, 1.8–1.9 µm Chamber controls used Minimal decrease in body weight at high dose in second yr Survival unaltered No increases in tumour incidence in males

[a] All lung tumours were of avelolar/bronchiolar origin.
[b] All tumours were benign pheochromocytoma except one which was malignant in the low-dose group.
d, day or days; F, female; h, hour or hours; M, male; NS, not significant; wk, week or weeks; yr, year or years

of the alveolar epithelium. In male rats, though treatment-related tumours were not observed, a dose-related increase in the incidence of atypical hyperplasia of the lung alveolar epithelium occurred. Atypical hyperplasia of the lung alveolar epithelium is considered potentially preneoplastic. In the female rats, dose-related increases in the incidence of adrenal medulla pheochromocytomas and an increase in mononuclear cell leukaemia at the highest dose were also reported (NTP, 2000).

3.6.3 Hamster

Another study using intratracheal instillation of gallium arsenide in hamsters (Ohyama *et al.*, 1988) was judged inadequate due to critical design flaws (short duration, small groups, etc.) with no indication of tumours.

3.7 Synthesis

Oral administration of sodium arsenate and DMA^{V} induced lung tumours in mice. Calcium arsenate induced lung tumours in hamsters by oral and intratracheal administration. Pre- and postnatal exposure in mice to arsenic trioxide, through subcutaneous injections (maternal and postnatal), induced lung tumours in the offspring. Transplacental exposure via maternal oral exposure in mice to sodium arsenite during gestation induced lung, liver, ovary and adrenal tumours in the offspring in several studies, and the uterus in one study. Early life transplacental and perinatal exposure to sodium arsenite appears to be a time of particular sensitivity in terms of carcinogenesis.

Oral exposure to DMA^{V} induced urinary bladder tumours in several studies in rats and among studies in mice, only one showed negative results. Oral trimethylarsine induced liver tumours in rats. Chronic oral exposure to MMA^{V} did not produce tumours in rats and mice. [The Working Group considered that previous traditional bioassays for arsenicals for adult rodents were frequently negative in their final evaluations.]

Inhalation of gallium arsenide causes lung and adenal tumours in rats but not in mice.

In multiple studies, initiating, promoting or co-carcinogenic activity was demonstrated in the urinary bladder, skin, female reproductive tract, kidney, lung, liver and thyroid after exposure to inorganic arsenicals or DMA^{V} in drinking-water or by transplacental exposure.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Most inorganic arsenic compounds are readily absorbed after oral exposure (about 80–90% for soluble compounds, and a smaller percentage for less soluble compounds), less well absorbed after inhalation (better for small particulates and soluble arsenicals), and least well absorbed after dermal exposure (NRC, 1999; IARC, 2004). Large airborne arsenic-containing particulates that are deposited in the upper airways may also be absorbed in the intestine if they are later swallowed. Hamsters exposed to gallium arsenide by the oral route or by intratracheal instillation showed the presence of As^{III} in blood and urine, but the majority of the gallium arsenide was excreted in faeces, indicating that absorption was limited by its insolubility. Absorption was about 30 times higher after intratracheal installation than by the oral route (Carter *et al.*, 2003).

The transport of As^{V} is thought to take place via phosphate transporters (Csanaky & Gregus, 2001). The sodium-coupled phosphate transporter NaPi-IIb may be responsible in part for the intestinal and hepatic uptake of As^{V} (Villa-Bellosta & Sorribas, 2008). As^{III} enters the cell by aquaglyceroporins 9 and 7 (Liu *et al.*, 2004),